Abstracts of
Royan International Twin Congress
9th Congress on Stem Cell Biology and Technology
4-6 September 2013

Royan Institute
Cell Science Research Center

Tehran, Islamic Republic of Iran
Aims and Scope: The "Cell Journal (Yakhteh)" is a publication of Royan Institute. It is published in English. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on exclusively cellular, molecular and other related topics. Cell Journal (Yakhteh) has been certified as a quarterly publication by Ministry of Culture and Islamic Guidance in 1999 and was accredited as a scientific and research journal by Health and Biomedical Information (HBI) Journal Accreditation Commission in 2000. This journal is a member of the Committee on Publication Ethics (COPE). This journal accepts (Original articles, Review articles, Short communications, Case reports, Editorial, Images in biology and Letter to editor) in the field of cellular and molecular science.

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The articles could be considered for publications in Cell Journal (Yakhteh) as below:
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B. Review articles are the articles by well experienced authors and those who have excellencies in the related fields. These are usually solicited by the editors, but we will consider unsolicited materials too. They will undergo editorial process except solicited articles. The corresponding author of the review article must be one of the authors of at least three articles appearing in the references. The review article consists of English Abstract (unstructured), Introduction, Discussion, Conclusion and References.
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2. Submission Process
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Page 2: Running title; a maximum of 7 words (no more than 50 characters).
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Results: They must be presented in the form of text, tables and figures. The contents of the tables should not be all repeated in the text. Tables and figures must be numbered consecutively as appeared in the text and should be organized in separate pages at the end of article while their location should be mentioned in the main text. Long articles may need sub-headings within some sections (especially the Results and Discussion parts) to clarify their contents.
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Conclusion: Emphasize the new and important aspects of the study. All conclusions are justified by the results of the study. It must be mentioned
Gone But not Forgotten
In the memory of the late Director of Royan Institute, Founder of Stem Cells Research in Iran and Chairman of *Cell Journal* (Yakhteh). May he rest in peace.

Dr. Saeed Kazemi Ashtiani

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Some of these abstracts have been previously published as full text in other journals. The authors will add more details and supplementary data to their presentations for more discussion in Royan International Twin Congress on Reproductive Biomedicine and Stem Cells Biology & Technology.
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Sahar Kiani

Dear Colleagues and Friends

On behalf of organizing committee, it’s my great pleasure and honor to welcome you to the 9th International Congress on Stem Cell Biology and Technology which is held accompanying with 14th congress on Reproductive Biomedicine (Royan International Twin Congresses), on September 4-6, 2013.

Regarding Royan Institute for Stem Cell Biology and Technology (RI-SCBT), Royan Institute as one of the premier institutes carrying out researches on Stem Cells, pursues the aim of Stem Cells and Developmental Biology for Regenerative Medicine all the time. Therefore RI-SCBT would like to play its role to make an opportunity for stem cell and biology scientists as well as physicians to debate and exchange their findings in a scientific and energetic atmosphere. Upon this goal, we have annually held this scientific meeting from 2005.

Increasing number of participants and received articles in this event indicates the fact of Stem Cells progression among basic and clinic professions. Every year, about 2000 participants including enthusiastic young researchers and principal investigators in Iran as well as in other countries, take part in this annual event. Moreover, expert scientists in Stem Cells from all over the world talk about their achievements in this field during the program.

I encourage you to join us for the 9th International Congress on Stem Cell Biology and Technology on September 4-6, 2013. I am convinced that this meeting will provide participants with unique experiences like cultural encounters with Iranian tradition and history, as well as the exchange of latest scientific knowledge from Stem Cells Researchers (Biologists and Physicians) from the scientific world.

Hope to see you in Tehran, Iran

Yours Sincerely,

Sahar Kiani, Ph.D.
Congress Chairperson
Stem Cell Biology and Technology Congress
Is-1: Current Status of Stem Cell Therapy in Royan Institute

Aghdami N1,2

1. Department of Regenerative Biomedicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Corresponding Email: Nasser.aghdami@royaninstitute.org

Regenerative therapies and cell replacement have great perspectives in the treatment of degenerative disorders in which the function or structure of the affected tissues or organs deteriorate over time. Stem cells, both embryonic and adult, are evaluated as the potential substrates of cell therapy for tissue regeneration in such diseases. In fact, stem cells can be considered revolutionary in the field of medicine. However before using the cell therapy routinely in clinics their potentials have to be studied in clinical trials if there are a reliable data in animal models for safety and effectiveness.

The filed of stem cell therapy, had a relatively strong start in Royan institute, benefiting from institute scientific infrastructure. The first clinical trials, using autologous bone marrow derived stem cell, was conducted in patients with myocardial infarction at 2006 and it followed with cell therapy trials in the kidney, vascular, liver, skin, and eye; bone diseases; and neurological disorders as well as progressively proceeding to the final phases of safety and efficacy. Now, there are more than 30 registered clinical trials by Royan institute in clinicaltrials.gov site. Clinical studies in Royan are focused mainly on the use of bone marrow and tissue specific stem cells and there is no any clinical trial using embryonic or fetal stem cell.

This presentation reviews the current clinical outcomes about cell therapy in heart, bone and joint and skin disorders which are conducted in Royan institute.

Is-2: OCT4B1, A Novel Spliced Variant of OCT4, Is Highly Expressed in Gastric Cancer and Acts As An Antiapoptotic Factor

Asadi MH1, Mowla SJ1, Fathi F3, Aleyasin A2, Asadzadeh J1, Atias Y1

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Center for Cellular and Molecular Research, Kurdistan University of Medical Sciences, Tehran, Iran
3. Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran

Corresponding Email: hossein_a1359@yahoo.com

Objective: The octamer-binding transcription factor 4 (OCT4) is involved in regulating pluripotency and self-renewal maintenance of embryonic stem cells. Recently, misexpression of OCT4 has been also reported in some adult stem as well as cancer cells; a finding which is still controversial. In addition to the previously described spliced variants of the gene (e.g., OCT4A and OCT4B), we have recently identified a novel variant of the gene, designated as OCT4-B1.

Materials and Methods: In this study, we investigated a potential expression and function of OCT4B1 in a series of gastric cancer tissues and a gastric adenocarcinoma cell line, AGS. Using the Taqman real-time PCR approach. We have also analyzed the effects of OCT4B1 knock-down in AGS cell line treated with specific siRNA directed toward OCT4B1.

Results: We have detected the expression of OCT4B1 in tumors with no or much lower expression in marginal samples of the same patients (p < 0.002). Our data revealed that interfering with the expression of OCT4B1 caused profound changes in the morphology and cell cycle distribution of the cells. Furthermore, down-regulation of OCT4B1 significantly elevated the relative activity of caspase-3/caspase-7 and the rate of apoptosis in the cells (more than 30%).

Conclusion: All together, our findings suggest that OCT4B1 has a potential role in tumorigenesis of gastric cancer and candidates the variant as a new tumor marker with potential value in diagnosis and treatment of gastric cancer.

Keywords: OCT4B1, Cancer Stem Cells, Gastric Denocarcinoma, Apoptosis

Is-3: Human Pluripotent Stem Cells for Modeling Neurological Disorders

Brüstle O

Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany

Corresponding Email: oliver.bruestle@uni-bonn.de

Is-4: Neural Cell Programming for Biomedical Applications

Brüstle O

Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany

Corresponding Email: oliver.bruestle@uni-bonn.de

Is-5: Regulation of Emergency Hematopoiesis

Böttcher S, Gerosa R, Radpour R, Manz MG*

Division of Hematology, University Hospital Zurich, Zurich, Switzerland

Corresponding Email: Markus.Manz@usz.ch

Objective: Severe systemic infections evoke a number of characteristic clinical signs such as fever, neutrophilia and the appearance of immature myeloid precursors in the circulation (left-shift). This reflects well-regulated hematopoietic response program to en-
hance myeloid cell output during times of increased hematopoietic demand, a condition which is referred to as emergency myelopoiesis. Important molecular components of the emergency myelopoiesis cascade, such as cytokines and transcription factors involved, have been elucidated. However, the initial steps of emergency myelopoiesis involving pathogen recognition and translation into accelerated bone marrow (BM) myelopoiesis have only been inferred from findings on Toll-like receptor (TLR) expression on immature hematopoietic stem and progenitor cells (HSPCs) as well as on mature hematopoietic cells (e.g. macrophages). Accordingly, it has been assumed that both immature as well as mature hematopoietic cells are involved in sensing infection and inducing emergency myelopoiesis directly and indirectly, respectively. By generating reciprocal BM chimeric animals with TLR4-/- hematopoiesis on a wild-type (WT) non-hematopoietic background (TLR4-/-•WT mice) and WT hematopoiesis on a TLR4-/- nonhematopoietic background (WT•TLR4-/- mice), we demonstrated that LPS-Induced emergency myelopoiesis depends on TLR4-expressing nonhematopoietic cells (Boettcher et al., J Immunol. 2012 Jun 15;188(12):5824-8.). However, the precise identity and localization of the nonhematopoietic cell type crucial for sensing gramnegative infection-derived lipopolysaccharide (LPS) has remained elusive to date.

Materials and Methods: We now have addressed this fundamental question using BM transplantation and Cre-loxP recombination technology.

Results: BM chimeric mice with a myeloid differentiation primary response gene 88 (Myd88) - deficiency in the hematopoietic lineage (MYD88-/-•WT mice) showed a normal LPS response indistinguishable to control (WT•WT) mice, while knocked out Myd88 within the nonhematopoietic compartment (WT•MYD88-/- mice) led to a non-responsiveness towards LPS similar to controls (Myd88-/-•Myd88-/- mice). These results are in line with our earlier data, thus confirming the critical role of the TLR4/MYD88 pathway in nonhematopoietic cells for the induction of emergency myelopoiesis. In order to specifically delete TLR-MYD88-downstream signaling in various nonhematopoietic cells including BM Nestin+ mesenchymal stem cells (MSCs) and their progeny, perivascular cells, endothelial cells, and hepatocytes, we generated Nes-Cre;Myd88fl/fl, Pdgfr-Cre;Myd88fl/fl, Tek-Cre;Myd88fl/fl, and Alb-Cre;Myd88fl/fl mice, respectively. We observed a normal increase in the frequency of BM CD11b+Gr-1low immature myeloid precursors accompanied by a decrease of BM CD11b+Gr-1high mature myeloid cells upon LPS stimulation characteristic for efficient emergency myelopoiesis in Nes-Cre;Myd88fl/fl, Pdgfr-Cre;Myd88fl/fl, and Alb-Cre;Myd88fl/fl mice as compared to control mice. Furthermore, we measured highly-elevated plasma G-CSF levels in these mouse strains upon LPS injection. Hence, intact TLR signaling in mesenchymal stromal cells incl. Nestin+ MSCs, perivascular cells as well as hepatocytes is dispensable for induction of emergency myelopoiesis. Strikingly, Tek-Cre;Myd88fl/fl mice were completely non-responsive towards LPS stimulation as assessed by the above-mentioned parameters.

Conclusion: Our results thus demonstrate a fundamental and unanticipated role of the endothelium for sensing of systemically spread pathogens and subsequent stimulation of BM emergency myelopoiesis.

Is-6: Clinical MRI Cell Tracking: The First 7 Years

Bulte JWM

Department of Radiology and Radiological Science, Division of MR Research, Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Corresponding Email: jwmbulte@mri.jhu.edu

Translational cellular imaging is expected to play a key role in evaluating the outcome of clinical trials using stem cells for tissue regeneration. In order to facilitate and implement the translation of novel experimental stem cell therapies into the clinic, one needs to be able to monitor the cellular biodistribution non-invasively following administration. Among the different clinically used cellular imaging techniques, 111In oxine scintigraphy is the only FDA-approved method and has been used primarily for imaging of infection and inflammation. Cellular magnetic resonance (MR) imaging, with its superior spatial resolution and excellent soft tissue anatomical detail, is emerging as the technique of choice to monitor in real-time image-guided stem cell delivery, immediate engraftment, and short-term homing. Up until now, 7 clinical MRI cell tracking studies have been published, all using superparamagnetic iron oxide nanoparticles or SPIOs in an off-label fashion. SPIOs are clinically approved and create strong local magnetic field disturbances that spoil the MR signal leading to hypo- or hyperintense contrast. A major setback is that the particles that were being used have been taken off the market, as their primary, FDA-approved indication (liver imaging of Kupffer cells) did not live up to its promise. However, several companies have started manufacturing novel particles, which possibly can also be used for magnetic particle imaging (MPI). Several other cellular imaging techniques are available, some of which are based on reporter genes, e.g. firefly luciferase for bioluminescent imaging, and herpes simplex virus thymidine kinase for positron emission tomography imaging. While the former cannot be used clinically because of physico-optical constraints, the latter has now also entered the clinic.

Is-7: General Principles and Methods for Non-Invasive Stem Cell Imaging

Bulte JWM

Department of Radiology and Radiological Science, Division of MR Research, Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
Two different methods exist to label stem cells with markers that can be detected by clinically applicable imaging methods. These are 1) labeling them with contrast agents and 2) transfecting them with a reporter gene. The formulations for direct labeling include positive (gadolinium-based) and negative (superparamagnetic iron oxide-based) contrast agents for 1H MR imaging, perfluorocarbons for 19F “hot spot” MR imaging, nanoparticles doped with positron emitters, i.e., gallium-68 or copper-64 for PET imaging, 111In-oxine for nuclear scintigraphy, iodine or gold particles for X-ray/CT imaging, and gas-filled bubbles for ultrasound imaging.

The drawback of using direct labeling with exogenous contrast agents or tracers is that the label may become undetectable after a certain number of cell divisions, when the label becomes diluted amongst daughter cells. Another confounding issue is cell death. It is not possible to discriminate live from dead cells, and the results may be misinterpreted when surrounding host cells (i.e., macrophages) take up the label and start migrating. Using reporter genes can solve some of these issues (i.e., only live cells will produce the reporter, which should be evenly replicated amongst daughter cells), and also offer the possibility of genetic manipulation to monitor if and when stem cells differentiate into downstream lineages. This can be done by placing the reporter gene under a specific promoter. However, whenever foreign proteins are introduced or overexpressed, there may be concern about potential immunogenicity and biological interference. Examples of reporter genes are the ferritin reporter for conventional MR imaging, lysine-rich GFP and the artificial lysine-rich protein for CEST MR imaging, the sodium iodide reporter for nuclear scintigraphy, and the HSV-tk reporter for PET imaging. Only the MR imaging reporter genes can be used without injecting a probe that binds the reporter.

Is-8: Scaffold Design for the Reduction of Host Tissue Response for Regenerative Medicine

Khang G

Department of BIN Fusion Technology and Polymer-Nano Science and Technology, Chonbuk National University, Jeonju, Korea
Corresponding Email: gskhang@chonbuk.ac.kr

Implanted biomaterials and drug delivery vehicles have been reported to induce sequential events of immunologic reactions in response to injury caused by implantation procedures and result in acute inflammation marked by a dense infiltration of inflammation-mediating cells at the materials-tissue interface. Poly (lactide-co-glycolide) (PLGA) is a member of a group of poly (α-hydroxy acid) that is among the few synthetic polymers approved for human clinical use by FDA. Consequently, it has been extensively used and tested for scaffold materials as a bioerodible material due to good biocompatibility, relatively good mechanical property, lower toxicity and controllable biodegradability. PLGA degrades by nonspecific hydrolytic scission of their ester bonds into their original monomer, lactic acid and glycolic acid. During these processes, there is very minimal systemic toxicity, however, in some cases, their acidic degradation products can decrease the pH in the surrounding tissue that result in local inflammatory reaction and potentially poor tissue development.

Currently, biomaterials are endowed with biocompatibility through three different methods which are: coating with hydrophilic molecules, modifying surface characteristics using physiochemical methods and impregnating bioactive substances. In our laboratory, the natural/synthetic nano-hybrid scaffolds have been investigated such as small intestine submucosa (SIS), demineralized bone particles (DBP), DBP gel, fibrin, keratin, hyaluronic acid, collagen gel, silk and a 2-methacyloxyloxyethyl phosphorylcholine (MPC) polymer (PMEH) polymer (PMEH) polymer (PMEH) polymer with PLGA to reduce cellular inflammatory response. In this lecture, we introduced synthetic/natural nanohybrid as DBP/PLGA and SIS/PLGA scaffold in terms of scaffold design for the reduction of host response and the augmentation of tissue formation. This information will be supporting the basic strategy for the scaffold design with better improved biocompatibility.


1. Division of Hematology/Oncology, Department of Medicine
2. Longwood Small Animal Imaging Facility, and Department of Radiology, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA
3. WCU Department of BIN Fusion Technology, Chonbuk National University, Jeonju 561-756, South Korea
4. Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC 27101, USA
Corresponding Email: gskhang@chonbuk.ac.kr

Conventional techniques for evaluating scaffold degradation demand large sample numbers and sacrificing animals at each time point, and this results in inaccurate conclusions due to their nonconsecutive single-time-point information. Therefore, there is a desperate need for a facile and noninvasive analytical method to continuously monitor scaffold degradation and tissue formation. In this study, we have developed a functional biodegradable scaffold to investigate matrix degradation by employing invisible near-infrared (NIR) fluorescence. Briefly, an 800 nm NIR fluorescence emitting ZW800-1 was conjugated on the collagen scaffold that was made from small intestinal submucosa using conventional N-hydroxysuccinimide (NHS) ester chemistry. Degradation behaviors and tissue formation were...
monitored noninvasively in one animal over 4 weeks using fluorescence imaging and magnetic resonance imaging (MRI), respectively. To quantify the degradation pattern, conventional analytical methods such as histological and gravimetric assays were also carried out at the same time. In addition, we also developed an optimal condition for \textit{in vitro} biodegradation studies, which can predict \textit{in vivo} tissue formation along with scaffold degradation. This noninvasive method using NIR optical imaging and MRI will be a benefit to tissue engineers and save time, reduce the use of animals, and offer more accurate conclusions.

**Is-10: Ethical Issues of Cell Therapy**

Larijani B

Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences, Tehran, Iran

Corresponding Email: emrc@tums.ac.ir

Cell therapy is a term used to describe the process of introducing new cells into a tissue for the purpose of treatment of different diseases. Although it is effective in treatment of many diseases, ethical issues are raised regarding its use. It is important to note that differences in moral values, cultural backgrounds, and religious principles can affect the decision of medical practitioners and policy-makers regarding the use of this new treatment option. Today, we aim to discuss some of the ethical considerations and challenges in regard with the use of stem-cell therapy. Several ethical issues need to be taken into account regarding Stem-cell therapy, including:

- The human rights of the unborn
- The use of a woman as an instrument to produce ova
- Creation of embryos (instead of using the spare ones)
- Relative advantages and risks of the practice
- The potential of the risk of creation of a commercial market for human cells
- The principle of subsidiary: to use cell-therapy only if there is no other alternative
- Resource allocation, justice, and conflict of interest
- Patenting stem-cell lines
- The risk of formation of animal-human hybrids

It can be concluded that despite all controversies surrounding the issue, some basic principles should be taken into account regarding the application of stem-cell therapy, such as:

- Protection of healthy individuals from the possible risks should be in priority
- Public confidence needs to be secured so that we can continue research in the field
- Standards for safety and efficacy of treatment should be set
- Ethical guidelines for donation, procurement, preservation and distribution of cells should be established
- Regional networks should be created for ethical considerations as socio-cultural backgrounds are similar in each region of the world.

In the end, we should keep in mind that the field of stem-cell therapy is still young and further research needs to be carried out in order to shed more light on its ethical considerations.

**Is-11: Cell-Based Regenerative Therapy As An Alternative to Liver Transplantation for End-Stage Liver Disease, Experience from Iran**

Malekzadeh R

Digestive Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran

Corresponding Email: malek@tums.ac.ir

Several types of cells including mature hepatocytes, adult liver progenitor cells and human embryonic stem cells, fetal liver progenitor cells, bone marrow-derived hematopoietic or mesenchymal stem cells, umbilical cord blood cells both in rodent and human have been reported to be capable of self-replication, giving rise to hepatocyte-like cells both \textit{in vivo} and \textit{in vitro}. Transplantation of these cells or their derivatives offer exciting promise for future treatment of cirrhosis and metabolic liver diseases, but significant technical hurdles remains that will only be overcome through years of intensive research. There is also serious concern about the long term safety of stem cell therapy and the possibility of tumor development. Here, we summarize our experience with cell therapy in treatment of chronic liver disease in Iran.

**Keywords:** Stem Cell, Liver Transplant, Cirrhosis, Cell Therapy, Bone Marrow

**Is-12: Consent in Cord Blood Banking and Its Applications**

Omani Samani R$^{1,2}$

1. Department of Epidemiology and Reproductive Health at Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Medical Ethics and History of Medicine Research Center, Tehran University of Medical Science, Tehran, Iran

Corresponding Email: samani@royaninstitute.org

The first time usage of Stem cells from umbilical cord in transplant medicine was reported in 1988. Over the next years, the use of cells from cord blood was accepted to be an effective alternative to cells from bone marrow in treatments for some malignant and metabolic diseases. So far, majority of cord blood transplants have been carried out from HLA matched unrelated donors. Cord blood banks are two types: private in which, people save their child's cord blood for the future use and pay the expenses and public in which, people donate their cord bloods to use for HLA matched patients. There are some ethical issues regarding cord blood bank both in banking and utilization of the cells. The
major ethical issue in this regard is "informed consent". The first argument is who owns the cord blood: father, mother or the child? Many countries relate the cord blood to the mother as the placenta is considered a part of her. But there are some claims that consider the child as the owner of cord blood, as it is genetically related to the child. In Iranian code of research ethics, for performing researches on cord blood, obtaining consent from both mother and father is considered necessary. But in the cord blood banks it remained uncertain, from mother, from father or from both. As the placenta has blood relation to the mother and the procedure of obtaining the cord blood involves mother alone, it seems that the consent should be obtained from mother alone. Perhaps, the same logic is used for obtaining consent for therapeutic abortion in Iran law, which needs the consent from mother alone. But, remaining question is for using the cells as a treatment for HLA matched patients, in the case that the child is grown up, would it be necessary to obtain the consent from the child or not? It seems that if the child is 16 years old or more, and the blood can be traced, the consent is necessary, but if the cord blood bank is anonymous, no need for it.

The second argument is about the information which is needed for the client of the private bank. There is no report about the potential harm for the mother although technician errors can occur. The fact that the culture of these cells has not been achieved and each blood could be used just for a 20 kg child should be discussed. The uncertain length of cryopreservation and possible usage for the child and family members must be fully presented. Insurance of the bank and possible harms to the bloods by accidents, fire and natural disaster should be mentioned. For public banks, the request of the donors is the priority to use the blood in exchange of their donation, which is impossible in most of the cases.

Finally, a detail contract must be written to clarify everything for the parties and avoiding any misunderstanding. Keywords: Cord Blood Bank, Private, Public, Ethics, Contract

**Is-13: Decidual Stromal Cells from Placenta - Experimental and Clinical Studies**

Ringdén O*, Kaipe H, Erkers T, Nava S, Molldén P, Sadeghi B

Division of Therapeutic Immunology and Center for Allogeneic Stem Cell Transplantation, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

Corresponding Email: Olle.Ringden@ki.se

**Objective:** One of the most serious complications after hematopoietic stem cell transplantation is acute graft-versus-host disease (GVHD), which develops when donor-derived T-cells recognize and become activated by alloreactive recipient tissue. We were the first to use bone marrow-derived mesenchymal stem cells (BM-MSCs) to reverse severe steroid-refractory acute GVHD and hemorrhagic cystitis. The fetus is protected against the mother’s immune system by the fetal membrane. We wanted to explore if stromal cells from the placenta could be used for GVHD and tissue damage in a similar way as MSCs.

**Materials and Methods:** We collected stromal cells from various parts of the placenta. To characterize these cells, we used fluorescence activated cell sorter (FACS). For functional analysis, mixed lymphocyte culture (MLC) and mitogen stimulation of human and mouse cells were used. For studies of GVHD, we used B6 mice as donors and Balb/C mice as recipients of hematopoietic stem cell transplants. Patients with steroid-refractory acute GVHD were also included.

**Results:** Stromal cells from the fetal membrane were easy to expand in vitro. We found that decidual stromal cells (DSCs) had the best immunosuppressive effect in vitro in MLC. Caryotyping of DSCs showed normal female karyotype. PCR showed that these cells were of female, maternal type. DCSs were positive for typical MSC markers, CD29, CD44, CD73, CD90 and CD105. DSCs were negative for hematopoietic markers, endothelial markers and epithelial markers. Unlike BM-MSCs, they were positive for PD-L1 and PD-L2 (negative regulation of co-stimulatory molecules). DSCs were also positive for CD49D, a marker for homing to inflammatory tissue (integrin). Indoleamine2,3-deoxygenase, prostaglandin E2, PD-L1 and interferon-γ seemed to participate in the immunosuppressive mechanism by DSCs. DSCs promote CD4+ CD25+ FoxP3+ regulatory T-cell expansion in a contact-dependent manner. Human DSCs inhibit mice MLC and also mice cells stimulated by phytohemagglutinin. Human DSCs also inhibited mice cells stimulated by human blood lymphocytes. For Balb/C mice undergoing stem cell transplantation with B6 donors, GVHD was reduced if DSCs were added on day 3 after transplantation. Human DSCs also stimulated proliferation in xeno-immunized mice, but could also depress this reactivity in vitro. Among eight evaluable patients with severe steroid-refractory acute GVHD, two had a complete response, four had a partial response with an overall response rate of 75%. Two patients did not respond at all. Melena stopped in three patients with severe hemorrhagic diarrhea after treatment with DSCs. Among the patients treated with DSCs, three are long-term survivors.

**Conclusion:** Stromal cells from the fetal membrane inhibit alloreactivity in vitro in MLC. They are of maternal origin, are safe to infuse to patients and they can inhibit and resolve GVHD in experimental animals and in patients. DSC therapy needs to be improved.

**Is-14: Differentiation, Dedifferentiation and Transdifferentiation Potential and Mechanisms of Human Bone Marrow-Derived Mesenchymal Stem Cells**

Ringe J*, Uliah M, Sittinger M

Tissue Engineering Laboratory and Berlin-Brandenburg Center for Regenerative Therapies, Dept. of Rheumatology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

Corresponding Email: jochen.ringe@charite.de
Objective: Mesenchymal stem cells (MSC) have a multilineage differentiation potential. Some years ago it was common opinion that during differentiation MSC become lineage restricted and unipotent in an irreversible manner. However, current data imply that even terminally differentiated cells transdifferentiate across lineage boundaries, and thus serve as progenitors for other lineages. This raises the questions of whether transdifferentiation occurs via dedifferentiation to a progenitor cell and subsequent differentiation or via direct cell-to-cell conversion, and whether the potency of MSC decreases or increases during differentiation. In this lecture, we will present the state of the art and own results on human MSC differentiation, de- and transdifferentiation in context of regenerative medicine. The focus lies on adipogenesis and subsequent de-/transdifferentiation.

Methods and Materials: Human MSC were isolated from bone marrow aspirates and expanded up to passage 4. Then, they were differentiated into the adipogenic (15 days), osteogenic (28 days) and chondrogenic lineage (28 days). Differentiated cells were isolated from their matrix and then dedifferentiated for 2-4 weeks in MSC expansion medium. Subsequently, dedifferentiated cells of all 3 lineages were again differentiated into 3 lineages. To analyze transdifferentiation via direct cell-to-cell conversion, in a further approach, differentiated cells were cultivated in differentiation medium of the other lineages. In both approaches, also single cell analysis was performed. In general, cultures were studied using histology, immunohistochemical staining, FACS, qPCR and GeneChips. Bioinformatics was performed to identify molecular key-players.

Results: GeneChip analysis in combination with Bioinformatics revealed a deep insight into adipogenic, osteogenic and chondrogenic differentiation and dedifferentiation. As shown here for adipogenic differentiation and dedifferentiation, we detected distinct genes whose upregulation (DHCR24, G0S2, MAP2K6, SESN3) and downregulation (DST, KAT2, MLL5, RB1, SMAD3, ZAK) is associated with cell cycle arrest in differentiated cells and perhaps narrow down the lineage potency. Upregulation of CCND1, CHEK, HGF, HMGA2 and downregulation of CCGG1, RASSF4, RGS2 is associated with cell cycle progression and maybe motivates dedifferentiation of differentiated cells. Interestingly, and of potential high importance for cell-based regenerative medicine, we found that the dedifferentiated cells of all three lineages have a multilineage potency comparable to MSC, and also observed an associative role of proliferation genes with cell cycle arrest and progression. Direct cultivation of adipogenic differentiated MSC in osteogenic medium resulted in a mixture of both cell types, as shown by staining of lipid droplets and of mineralized bone matrix, and qPCR of adipogenic (PPARG, FABP4) and osteogenic (SPP1, RUNX2) marker genes. Mix cultures were also observed in chondrogenic medium (PPARG, FABP4, SOX9, COL2A1 positive).

Conclusion: Our results indicate that transdifferentiation of differentiated MSC proceeds via dedifferentiation and correlates with cell cycle arresting and driving genes. Understanding of de-/transdifferentiation mechanisms may allow the use of cells from easily available cell sources in regenerative medicine. Our results will be presented in this keynote in context of the international state of the art.

Keywords: Mesenchymal Stem Cells, Differentiation, Dedifferentiation, Transdifferentiation, Regenerative Medicine

Is-15: Migration and Differentiation Capacity of Mesenchymal Stem Cells from Patients with Osteoarthritis - Towards In Situ Joint Cartilage Tissue Engineering

Ringe J1*, Dehne T1, Andreas K1, Naderi-Meshkin H2, Ullah M1, Gulich K1, Sittinger M1

1. Tissue Engineering Laboratory & Berlin-Brandenburg Center for Regenerative Therapies, Department of Rheumatology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
2. Department of Biology, Ferdowsi University of Mashhad, Mashhad, Iran

Corresponding Email: jochen.ringe@charite.de

Objective: Today, for the regenerative treatment of injured or early osteoarthritic (OA) cartilage, autologous chondrocyte implantation (ACI) or matrix assisted ACI (MACI) are clinically applied. Also mesenchymal stem cell (MSC) approaches have reached the clinic, combining microfracture with the implantation of a collagen membrane (AMIC) or PGA/hyaluronan scaffold (Chondrotissue). AMIC is a passive approach; endogenous MSC flow into the membrane. Chondrotissue, in whose development we were involved, is between passive and active; MSC flow into the scaffold is enhanced by the addition of MSC recruiting serum. Our aim is to develop an active in situ approach, wherein the implantation of scaffolds loaded with MSC recruiting chemokines (CK) and differentiation factors, combined with microfracture, allows the use of endogenous MSC for cartilage regeneration. In this lecture, we will present the international state of the art and our own results.

Materials and Methods: Bone marrow MSC of normal donors (ND) and OA patients were characterized (growth kinetics, FACS CD marker profile, multilineage assays). Also their CK receptor profile (qPCR, antibodies), CK release profile (protein array) and CK dependent migration (chemotaxis assays) was elucidated. Moreover, expression profiles of CK stimulated ND and OA MSC were compared (microarrays). Selected CK were encapsulated in PLGA release particles, and in vitro and in vivo applied to attract superparamagnetic iron oxide nanoparticle (SPION) labeled MSC (rat model, MRI). Finally, comparative gene expression profiling was performed for ND and OA MSC fibroigen/PLGA implants after chondrogenic induction.

Results: ND and OA MSC have a similar doubling...
time, differentiate to fat, bone and cartilage, are CD73, CD105 positive, and CD45 negative. Moreover, both express most CK receptors. Proteomics revealed similar CK release profiles. Based on 96-well chemotaxis assays, they also show a similar CK dependent migration potential. Promising CK like CCL25 (TECK) and CXCL12 (SDF1•) were analyzed in more detail. CCL25 recruited a high number of ND and OA MSC and represents a promising new candidate for in situ approaches. Expression profiling of CCL25 and CXCL12 induced ND and OA MSC gave us a deep insight into their mobilization. For example, 22 genes were differentially expressed in both ND and OA MSC. Most are involved in homing (PDE4B), movement (PTGS2) and cytoskeletal and membrane reorganization (IGFBP1). In an ongoing study, CCL25 loaded PLGA particles are tested in a rat model. Here, for in vivo MRI monitoring of MSC migration towards CK releasing particles, SPION labeled MSC are used. Finally, the regenerative potential of OA and ND MSC was studied in fibrinogen/PLGA transplants. Here, chondrogenesis resulted in fibro- and joint cartilage, and ND and OA expression profiles showed a similar expression of marker genes known in context of OA (COL10A1, MMP1 and -3).

Conclusion: We have shown that end-stage OA MSC behave like ND MSC, and that we have established the key knowledge and tools for an active, MSC based in situ therapy of injured or OA joint cartilage. This will be presented in this keynote in context of the international state of the art.

Keywords: Mesenchymal Stem Cells, Migration, In Situ Tissue Engineering, Osteoarthritic Cartilage

**Is-16: Immunomodulatory Effects of Mesenchymal Stem Cells and Use in Allogeneic Hematopoietic Stem Cell Transplantation**

**Ringdén O**

Division of Therapeutic Immunology and Center for Allogeneic Stem Cell Transplantation, Karolinska Institutet, Stockholm, Sweden

**Corresponding Email: Olle.Ringden@ki.se**

**Objective:** Mesenchymal stem cells (MSC) can differentiate into several tissues, including bone, cartilage and fat, in vitro and in vivo. MSC may be important in regenerative medicine for tissue repair.

**Materials and Methods:** In vitro, we studied MSC in mixed lymphocyte culture (MLC), mitogenic stimulation and in cell-mediated lympholysis with chromium-labelled target cells. In vivo, MSC were studied in patients with acute and chronic graft-versus-host disease (GVHD), hemorrhagic cystitis and hemorrhages following hematopoietic stem cell transplantation.

**Results:** MSC have low immunogenicity and immunomodulatory effects. In vitro, MSC suppress alloantigen-induced T-cell functions in vitro in mixed lymphocyte culture (MLC) and cytotoxic T-cells (CTL). This effect was seen regardless of HLA compatibility between MSC and responder or stimulatory cells in alloreactivity. Pooling of MSC from several donors generated higher and more stable suppression in both MLC and after mitogenic stimulation. We have found that MSC from different individuals have varying capacity to inhibit alloreactivity. Furthermore, single clones from the same individual also differ regarding capacity to induce immunosuppression. Suppressive MSC also seems to differ from non-suppressive MSC regarding the phenotype and gene profiles. We found that Epstein-Barr virus and cytomegalovirus induced proliferation and interferon-γ (IFNγ) production from blood lymphocytes was less affected by third party MSC than response to alloantigen. Furthermore, MSC had no effect on expansion of EBV and CMV pentamer specific T-cells. This suggests that the effect of functions of virus specific T-cells may be retained after MSC infusion. Clinically, we found that MSC can reverse severe acute graft-versus-host disease (GVHD). MSC were found to home to target organs of GVHD. In 55 allogeneic hematopoietic stem cell transplant patients treated for severe acute GVHD, 30 patients had a complete response and survival was 52%. For patients with partial or no response (n=25), 2-year survival was 15% (p=0.02). MSC showed positive effects in autoimmune disease models. Because chronic GVHD resembles autoimmune disorders, MSC were used also to treat chronic GVHD. Response to MSC therapy was seen in around half of the patients with chronic GVHD. We have also used MSC for tissue repair, such as hemorrhagic cystitis and perforated colon in allogeneic HSCT patients. MSC also interfere with coagulation and were found to stop major hemorrhages following stem cell transplantation. MSC have also been used to enhance engraftment in HSCT patients and for graft failure.

**Conclusion:** Possible clinical applications to the immunomodulatory and tissue repairing effects of MSC include acute and chronic GVHD, tissue repair, treatment of rejection of organ allografts, hemorrhages and autoimmune disorders.

**Is-17: Enhancing Mammalian Regeneration**

**Rosenthal NA**

1. Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia
2. National Heart and Lung Institute, Imperial College London, London, United Kingdom

**Corresponding Email: nadia.rosenthal@monash.edu**

**Objective:** What lies behind the remarkable potential of some organisms to rebuild themselves after injury, and why aren’t mammals better at it? The limited restorative capacity of human tissues has been attributed to the loss of adequate cell replacement coupled with persistent inflammation with increasing age. Our imaginations have been captivated by mounting evidence for populations of stem cells in adult tissues, perhaps set aside earlier in the embryo, that might be coerced into regenerative
service in later life. Stem cells have attracted huge scientific and public interest, not only because they bear the promise of miracle cures for age-related diseases, but also because their medical use is so appealing: stem-cell therapy would augment the human body’s own regenerative capacity, which declines as we grow older.

**Materials and Methods**: Our approach has been to develop mouse models to tinker with mechanisms at work in the mammalian response to damage, disease and ageing, by reducing the impediments to effective regeneration. The laboratory mouse is widely considered the model organism of choice for studying the diseases of humans, from whom they differ in only a tiny fraction of their genetic material. A distinguished history of classical genetic experimentation in the mouse has recently gathered speed with the advent of powerful new tools to manipulate the murine genome. The recent launch of several internationally sponsored initiatives for systematic mouse mutagenesis on a large scale using various genetics strategies, along with high throughput phenotyping pipelines, underscores the utility of the mouse for interpreting the mammalian genome, and for generating increasingly more accurate models of human disease.

**Results**: Interventions in growth factor delivery using mouse models support the feasibility of recapitulating regenerative capacity by modulating key signaling pathways to restore injured or degenerating mammalian tissues. In each model, distinct cellular components are employed, providing new targets for clinical intervention.

**Conclusion**: The appropriate source of cells for therapeutic applications in regenerative medicine is hotly debated, and much work must be done before stem cell therapy can become a medical reality; even the recent explosion of information on stem cell pluripotency has still not brought us a clear understanding of the underlying biological processes in any system. Other mechanisms at work in the normal regeneration process may be more successfully harnessed to increase the efficiency of stem cell-mediated regeneration.

**Is-18: Vertebrate Regeneration and The Immune System**

Rosenthal NA1,2

1. Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia
2. National Heart and Lung Institute, Imperial College London, London, United Kingdom

**Corresponding Email**: nadia.rosenthal@monash.edu

**Objective**: The adult mammalian body does retain the robust repair capacity of embryonic stages. In contrast to the effective regeneration of other vertebrates, the limited restorative capacity of many adult mammalian tissues has been attributed to the loss of adequate cell replacement coupled with persistent inflammation.

**Materials and Methods**: Using genetic manipulation we have investigated the role of growth factors and resident immune cells in the resolution of tissue injury, in both mouse and axolotl, an efficiently regenerating member of the urodele amphibian family.

**Results**: We have uncovered a complex interaction between local repair mechanisms and immune cells, which participate in the removal of necrotic tissue, secrete growth factors that limit inflammation, maintain tolerance and promote progenitor cell-mediated tissue replacement. We have recently discovered an unexpected connection between regenerative processes and immune tolerance.

**Conclusion**: Our work supports the feasibility of improving cardiac regenerative capacity by modulating key signaling pathways controlled by specific components of the immune system, providing new targets for clinical intervention and improving prospects for molecular and cellular combination therapies.

**Is-19: Impact of TLR-Agonists on Hematopoietic Stem Cell Homeostasis**

Takizawa H, Saito Y, Krottouyuk LV, Radpour R, Manz MG*

Division of Hematology, University Hospital Zurich, Zurich, Switzerland

**Corresponding Email**: Markus.Manz@usz.ch

**Objective**: Systemic bacterial infection triggers inflammatory responses, as exemplified by septic shock. While humoral mediators and mature hematopoietic cell reactions are well studied in this process, little is known about respective responses in immature hematopoietic cells in the bone marrow (BM). We have previously shown that short-term repetitive injection of lipopolysaccharide (LPS), a gram negative bacterial component, induces proliferation of dormant hematopoietic stem cells (HSCs) in BM that retain lifelong self-renewal and multi-lineage differentiation. Here we examine the underlying molecular pathways as well as cellular function of HSCs upon chronic LPS challenge.

**Materials and Methods**: Genetically modified mice which lack toll-like receptor 4 (TLR) or downstream signal molecules have been used to generate BM chimeric mice and were challenged with LPS purified from E. coli. 0111:B4.

**Results**: To dissect if TLR4 ligation by LPS leads to direct or indirect activation of HSCs, we generated mixed BM chimeric mice by reconstituting wild-type (WT) recipients with WT and Toll-like receptor 4 deficient (TLR4 KO) cells in a 1:1 ratio followed by systemic injection of PBS or LPS. Monthly peripheral blood analysis showed that in animals chronically challenged with LPS, TLR4 KO cells outcompeted WT cells in all lineages, while PBS treated controls maintained a 1 to 1 chimerism. The same result was observed when TLR4KO recipient mice were used. Secondary transplantation showed continuous decline in hematopoietic contribution of WT cells. These data demonstrate that direct activation of TLR4 on HSCs limits their competitive repopulating ability.
In mature immune cells TLR4 ligation by LPS activates two downstream pathways mediated by two proximal adaptor molecules, MYD88 and TRIF. To determine the respective pathways active in HSCs, we utilized TRIF and MYD88 KO mice. Acute LPS challenge induced up-regulation of Sca-1 expression and thus numeric expansion of immunophenotypically defined HSC populations in WT mice. This effect was absent in TRIF KO but not in MYD88 KO mice. Consistent with phenotypic changes, TRIF KO HSCs, similar to TLR4 KO HSCs, retained a competitive repopulating advantage over WT cells upon LPS stimulation, while this was not observed in MYD88 KO HSCs. However, the lack of response to LPS in TRIFKO is not due to general cellular unresponsiveness, as Pam3CSK4, a ligand for TLR1/2, causes both phenotypic expansion and dysfunction in TRIFKO HSCs. Furthermore, we observed rapid activation of reactive oxygen species (ROS) and p38 (mitogen activated protein kinase 14) in HSCs after in vivo LPS stimulation. Pharmacological inhibition of both molecules blocked phenotypic HSC (LKS) expansion in acutely LPS-treated mice, and rescued HSC from LPS-induced dysfunction in competitive repopulation experiments. In contrast, analysis of mature hematopoietic cells showed that early expansion of immature granulocytes in response to LPS was abrogated in MYD88 KO mice, while WT, TRIF KO, and WT mice treated with ROS/p38 inhibitors retained this response, indicating that emergency granulopoiesis is dependent on TLR4-MYD88 signaling.

**Conclusion:** Our findings demonstrate that LPS directly acts on TLR4 expressed on HSC in BM and causes functional impairment through TRIF-ROS-p38 signaling pathways. Thus, specific blockage of TRIF-ROS-p38 or further downstream signals during inflammation might allow to prevent HSCs from functional exhaustion, while preserving at least short-term production of granulocytes for an efficient innate immune response against pathogens.

**Is-20: The Epithelial to Mesenchymal Transition in Cancer and Organ Degeneration**

**Toledano MAN**

Institute of Neurociencias CSIC-UMH, 03550 San Juan de Alicante, Spain  
*Corresponding Email: anieto@umh.es*

The epithelial to mesenchymal transition (EMT) is required in the embryo for the formation of tissues which cells originate far from their final destination. The EMT endows cells with migratory and invasive properties. However, once the cells reach their destinations the EMT program must be downregulated. As such, the EMT inducers need to be kept silent in the adult to protect epithelial homeostasis and tissue architecture. The reactivation of the EMT programme in the adult promotes cancer progression and organ fibrosis and recent findings indicate that the EMT can also confer stem cell properties. As the EMT was recruited during evolution to define embryonic territories and to control epithelial plasticity, the embryo holds the clues to the molecular and cellular mechanisms operating after its reactivation in the adult, despite the peculiarities associated with different pathological EMTs. I will discuss recent findings using different model systems to show the contribution of the EMT to different pathologies. As such, when activated in cancer, it promotes the delamination of cells from the primary tumour, the first step of the metastatic cascade. On the other hand, the reactivation of the EMT in normal adult epithelial cells leads to development of organ fibrosis. In the kidney, fibrosis is the link between progressive loss of renal function and primary diseases such as glomerulonephritis, diabetes, toxic injury, congenital abnormalities, urinary tract obstruction and chronic rejection of transplanted kidneys. In the context of the reactivation of EMT, I will discuss the design of therapeutic strategies to fight both organ degenerative diseases and one of the most dangerous aspects of cancer, the formation of metastasis.

**Is-21: The Epithelial to Mesenchymal Transition in Embryonic Development**

**Toledano MAN**

Institute of Neurociencias CSIC-UMH, 03550 San Juan de Alicante, Spain  
*Corresponding Email: anieto@umh.es*

The epithelial to mesenchymal transition (EMT) converts epithelial cells into migratory and invasive cells and is a fundamental event in morphogenesis. Indeed, with the exception of ectodermal derivatives, notably the central nervous system and the epidermis, all vertebrate tissues are the result of one or several rounds of epithelial-mesenchymal transitions. In the embryo proper, the first EMT event occurs at gastrulation, when a subset of cells undergoes EMT to internalize and generate the mesoderm and the endoderm. Among the transcription factors that trigger bona fide EMTs, Snail genes are a good example to understand this process. Their role in development ranges from the subdivision of the early embryo into two main territories -ectodermal and mesendodermal- to the delamination of the neural crest and the formation of the heart valves. In addition to the induction of EMT, Snail can also control the cell proliferation and cell survival. I will discuss the roles of Snail genes during embryonic development and the effects of Snail deregulation both during the definition of embryonic territories and during organ development. As such, embryos with defective Snail at early stages cannot progress through gastulation and an excess of Snail function during bone development leads to achondroplasia, the most common form of dwarfism in humans.

**Is-22: Mesenchymal Stem Cells Shape Microglia**
Effector Functions through The Release of CX-3CL1

Uccelli A1, Parodi B1, Usai C2, Vergani L3, Casazza S1, Bruzzone S1, Mancardi G3, Giunti D1

1. Department of Neurology, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI) University of Genoa, Genoa, Italy
2. Institute of Biophysics, National Research Council, Genoa, Italy
3. DipTERIS, University of Genoa, Genoa, Italy
4. Department of Experimental Medicine, Section of Biochemistry, University of Genoa, Genoa, Italy
Corresponding Email: auccelli@neurologia.unige.it

Objective: Mesenchymal stem cells (MSCs) display a remarkable ability to modulate the immune response and protect the central nervous system (CNS) mainly through the release of soluble factors in a paracrine fashion, affecting the functional behavior of cells in the tissues. Here we investigated the effect of the interaction between MSC and microglia in vitro and we dissected the molecular and cellular mechanisms of this cross talk.

Materials and Methods: In this study, we addressed the in vitro effect of MSC on microglia and we dissected the molecular and cellular mechanisms of these interactions demonstrating that MSC can switch microglia from a detrimental behavior dominated by the release of pro-inflammatory molecules to a protective phenotype associated with the production of anti-inflammatory and trophic factors. Moreover we showed that MSC induce functional changes on microglia as depicted by modifications in intracellular calcium concentration and phagocytic activity. Finally we provided compelling evidence that CX3CL1 released by MSC plays a major role in inducing these beneficial effects on microglia.

Results: We demonstrated that MSC impair microglia activation by inflammatory cues through the inhibition of the expression and release of inflammatory molecules and stress associated proteins. We showed that MSC significantly increase microglial expression and release of molecules associated with a neuroprotective phenotype such as CX3CR1, NURR1, CD200R and IGF1. Interestingly MSC can enhance functional changes on microglia as depicted by the increase of intracellular calcium concentration and phagocytic activity. This last event is associated with an increased expression of TREM2, an innate immune receptor involved in phagocytosis in the absence of inflammation. The observed effects on CX3CR1 expression microglia are due to the release of CX3CL1 by MSC, driven by inflammatory signals, as demonstrated by the reversal of the observed results when CX3CL1 expression was silenced in MSC or its release was blocked. Last, we showed that exogenous CX3CL1 induce phenotypic and functional changes of microglia similar to those induced by MSC.

Conclusion: These findings demonstrate that MSC instruct, through the release of CX3CL1, microglia responsiveness to pro-inflammatory signals by modulating constitutive “calming” receptors, typically expressed by “steady-state microglia” thus switching microglia from a detrimental phenotype to a neuroprotective one.

Keywords: Mesenchymal Stem Cells, Microglia, Chemokines, Neuroprotection, Immunomodulation

Is-23: Construction of A Novel 3D Living Hyaline Cartilaginous Graft (LhCG) As In Vitro Endochondral Niches for Engineered Osteo/Chondrogenesis

Wang D

Division of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore
Corresponding Email: dawang@ntu.edu.sg

This study is designed and practiced for engineered articular regenerative medicine. The traditional approach is to mimic pre-cartilage condensation during primary development, that is, to first generate chondrocyte isogenic groups (colonies) via suspended pellet culture, then condense these chondrocytic pellets into scattered nodules-like microscopic cartilaginous tissues, and further assemble these pieces of micro-tissues into a single piece of tangible macro-scaled cartilaginous tissue. The disadvantage of this “stack-up” strategy is that the final cartilaginous tissue generated remains too small in size to be practically handled for any applications and in the process of scaling up from micro to macro tissue, it would inevitably cause various degrees of phenotype loss as well as introduction of impurities, typically, due to collagen type I related fibrosis.

In this study, we have innovatively developed a continuous methodology to directly set up a macro-scaled three-dimensional (3D) living hyaline cartilage graft (LhCG) with the aid of a biomaterial-based interim scaffolding system - alginate hydrogel in which hyaline chondrocytes are accommodated and guided to grow into scattered micro-tissues and further interconnecting them into a single piece of tangible macro-scaled cartilaginous tissue. The disadvantage of this “stack-up” strategy is that the final cartilaginous tissue generated remains too small in size to be practically handled for any applications and in the process of scaling up from micro to macro tissue, it would inevitably cause various degrees of phenotype loss as well as introduction of impurities, typically, due to collagen type I related fibrosis.

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or target at other tissue or organ regenerations. Given the established cartilaginous construction, we also explored the possibilities of using LhCG as a ready-made platform to mimic endochondral osteogenesis for bone formation. The results suggest that LhCG not only serves as a good living graft for cartilage regeneration, it is able to achieve significant ossification when directed toward osteogenesis via a simple switch in media composition.

The on-going research with LhCG is to introduce angiogenesis by hybridizing endothelial progenitor cells (EPCs) in pursuit of a complete ossification in situ. We believe that with capillary network in place in LhCG platform, the application of LhCG is not restricted to cartilage or bone regeneration; LhCG could be directed to many other tissue and organ regenerations.

Is-24: Engineering Chondrogenesis with Growth Factor and Antisense Co-Transgenic Synovial Mesenchymal Stem Cells (SMSCs)

Wang D

Division of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, Nanyang, Singapore

Corresponding Email: dawang@ntu.edu.sg

Articular diseases such as osteoarthritis are always a cause of suffering and pain among the elderly. Reparation and regeneration of articular cartilage tissues defected by ageing, disease or trauma have been an unmet need. The therapeutic efficacy of traditional treatments remains suboptimal. Hereby we propose to combine the recent developments in gene therapy and tissue engineering to provide a promising solution for this unmet challenge.

Synovial mesenchymal stem cells (SMSCs) have been recently found to be an excellent source for cell-based chondrogenesis due to its abundance, self-renewal ability and superiority in chondrogenesis. To successfully drive SMSCs to differentiate into chondrocytes, a member of the transforming growth factor superfamily, namely transforming growth factor β3 (TGF-β3), has been studied and found to be effective in the induction of chondrogenesis. In our previous study, SMSCs have also been successfully induced to differentiate into chondrocytes with released TGF-β3 mediated by recombinant adenovirus. However, one obstacle in chondrogenesis with SMSCs is that type 1 collagen is expressed in SMSCs, and simultaneously down-regulate the expression of either TGF-β3 or anti-type 1 collagen antisense targeting type 1 collagen were constructed. To fulfill the expected two functions simultaneously- that is, to express TGF-β3 and suppress type 1 collagen expression, we constructed a viral vector that merges the two functions into one vector, while also co-transduced with two single-functioning vectors that express TGF-β3 and type 1 collagen-targeted shRNA, respectively. To generate tissues for articular cartilage restoration, SMSCs transduced with various combinations of these viral vectors express promotional TGFβ3 to facilitate chondrogenic differentiation and growth of SMSCs, and simultaneously down-regulate the expression of type 1 collagen for better property and maintenance of the neo-tissue desired as articular hyaline cartilage. The various combinations of either recombinant adenovirus or lentivirus implement a controlled release/ expression of either TGF-β3 or anti-type 1 collagen shRNA in a transient or permanent manner. These viral vector transducing systems were applied coupling with a hydrogel scaffold based 3D culture system. By comparison between the various groups in terms of chondrogenic induction and type 1 collagen suppression, the optimal scheme is selected and established as a sound system for the engineering of a successful cartilage construct.
Conclusion: The lesion epicenter, and regions rostral and caudal to the lesion, showed reduced cavity formation and increased cell density at the site of injury. These cells incorporated into injured retina in a dose-dependent manner. The intravenous route resulted in optimal localization of donor cells at the site of injury. These cells incorporated into injured retina in a dose-dependent manner.

Results: The induced MNLCs were immunoreactive to Oligo2 and choline acetyltransferase. The results of the BBB test, relative difference coefficients (RDC), immunohistochemical staining, and morphometry (for 12 weeks after injury).

Materials and Methods: Adipose-derived stem cells (ADSCs) were preinduced with selegiline and then induced with Shh and all-trans retinoic acid (RA). The treated animals were evaluated using the Basso, Beattie, and Bresnahan (BBB) test, relative difference coefficients (RDC), immunohistochemical staining, and morphometry (for 12 weeks after injury). The induced MNLCs were immunoreactive to Oligo2 and choline acetyltransferase. The results of the BBB test showed a significant improvement in the group that received combination treatment with MNLCs and GDNF, as compared with the untreated group. Histological assessments showed that combination therapy reduced cavity formation and increased cell density at the lesion epicenter, and regions rostral and caudal to the lesion.

Conclusion: The conclusion of our study is that combination treatment with MNLC transplantation and GDNF ameliorates locomotor dysfunction in rats with SCI.

Keywords: Combined Therapy, GDNF, Spinal Cord Injury, ADSCs

Os-3: Recruitment of Stem Cells into The Injured Retina after Laser Injury

Anand A*, Singh T, Prabhakar S, Gupta A

Neuroscience Research Lab, Department of Neurology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

Corresponding Email: akshay2anand@gmail.com

Objective: Retinal degeneration is a devastating complication of diabetes and other disorders. Stem cell therapy for retinal degeneration has shown encouraging results but functional regeneration has not been yet achieved.

Materials and Methods: Our study was undertaken to evaluate the localization of stem cells delivered to the retina by intravenous versus intravitreal infusion, because stem cell localization is a key factor in ultimate in vivo function. We used lineage-negative bone marrow-derived stem cells in a model wherein retina of mice was induced by precise and reproducible laser injury. Lin(-ve) bone marrow cells (BMCs) were labeled with a tracking dye and their homing capacity was analyzed at time points after infusion.

Results: We found that Lin(-ve) BMCs get incorporated into laser-injured retina when transplanted through either the intravitreal or intravenous route. The intravenous route resulted in optimal localization of donor cells at the site of injury. These cells incorporated into injured retina in a dose-dependent manner.

Conclusion: The data presented in this study reflect the importance of dose and route for stem cell-based treatment designed to result in retinal regeneration.

Keywords: Retinal Stem Cells, Laser Injury, Retina, Regeneration

Os-4: Establishment of Pluripotent Embryonic Germ Cells by Inhibition of Signaling Pathways in a Chemically Defined Medium

Attari F1, 2*, Sepehri H1, Ansari H2, Asgari B3, Shahverdi A3, Baharvand H2, 4

1. Department of Animal Biology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Embryology at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Corresponding Email: baharvand@royaninstitute.org

Primordial germ cells (PGCs) are the unipotent cells...
with the ability to transform into a pluripotent state named as embryonic germ cells (EGCs) in vitro. EGC formation is more efficient, has a shorter culture period than somatic cell reprogramming and does not require exogenous genetic manipulation. Thus these cells are a suitable candidate to analyze mechanisms by which committed cells acquire a pluripotent state. In the present study we have attempted to elucidate a more defined, efficient protocol that derives EGCs by the suppression of TGF-β signaling and simultaneous inhibition of the ERK pathway. To achieve this, we applied two small molecules that inhibited the above mentioned pathways. Under this condition we noted that one day presence of stem cell factor (SCF) as a survival factor was adequate for PGC reprogramming, while the efficiency of this transformation was considerable. Moreover we have shown that this chemically supplied medium rules out the need for serum and feeder cell presence in culture. The resultant EGCs were characterized for pluripotency markers in gene and protein expression level and also for in vivo and in vitro differentiation capacity. Here we report that PGCs from different embryo ages can be reprogrammed into pluripotent stem cells through manipulating signaling pathways under chemically modified culture condition, regardless of animal genetic background.

**Os-5: Bone Tissue Engineering: Progress and Challenges**

Bagheri F*, Baghaban Eslaminejad MR

Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Corresponding Email: fatemeh_4117@yahoo.com

Regeneration of large bone defects is considered a challenging task by facio-mandibular and orthopedic surgeons. Bone tissue engineering has been viewed as a promise alternative to the conventional use of bone grafts, due to their limitless supply and no disease transmission. In bone tissue engineering, life science as well as engineering is involved to manufacture an appropriate bone construct. Building blocks for engineered constructs include biomaterials, cells, and growth factors. These factors would be applied alone or in combination depending on the nature of bone tissue defects. At the field of bone tissue engineering and regeneration, there are several challenging issues including the optimal cell source, chemical and physical features of materials and vascularization in replaced tissue. Many scientists believed that mesenchymal stem cells would be considered as an ideal cell source for bone tissue engineering owing to their unique properties. These cells could achieve reasonable regeneration in bone hard-to-cure defects if provided with an appropriate biomaterial and bioactive molecules capable of inducing both differentiation and vascularization.

**Keywords:** Bone Tissue Engineering, Clinical Challenges, Stem Cells, Scaffolds, Vascularization

**Os-6: Immunomodulation by Transplanted Human Embryonic Stem Cell-Derived Oligodendroglial Progenitors in Experimental Autoimmune Encephalomyelitis**


Institute for Cell Engineering, Johns Hopkins University, Baltimore, USA
Corresponding Email: jwbulte@mri.jhu.edu

**Objective:** Transplantation of embryonic stem cells and their neural derivatives can lead to amelioration of the disease symptoms of experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS). We sought to elucidate potential immunomodulating effects of transplanted cells and to investigate whether or not prolonged cell survival is a prerequisite to obtain such an effect.

**Materials and Methods:** Oligodendroglial progenitors (OPs), derived from human embryonic stem cells (hESC, HES-1), were labeled with superparamagnetic iron oxide and transduced with luciferase. At 7 days following induction of EAE in C57/BL6 mice, 1E6 cells were transplanted in the ventricles of C57/BL6 mice and noninvasively monitored by magnetic resonance and bioluminescence imaging.

**Results:** Cells were found to remain within the cerebroventricular system and did not survive for more than 10 days. However, EAE mice that received hESC-OPs showed a significant improvement in neurological disability scores (0.9±0.2; n=12) compared to that of control animals (3.3±0.4; n=12) at day 15 post-transplantation. Histopathologically, transplanted hESC-OPs generated TREM2-positive CD45 cells, increased TIMP-1 expression, confined inflammatory cells within the subarachnoid space, and gave rise to higher numbers of Foxp3-positive regulatory T cells in the spinal cord and spleen.

**Conclusion:** Despite the lack of prolonged cell survival, these results suggest that transplantation of hESC-OPs can alter the pathogenesis of EAE through immunomodulation, potentially providing new avenues for stem cell-based treatment of MS.

**Keywords:** Human Embryonic Stem Cells, Oligodendrocyte Progenitors, Transplantation, Multiple Sclerosis, Immunomodulation, Magnetic Resonance Imaging, Bioluminescent Imaging

**Os-7: 3. Neural Gene Expression in Embryonic Stem Cells Can Be Affected under Treatment of Alzheimer’s Patients Serum In Vitro**

Dehghani L1,2,*, Razaezadeh I, Meamar R1, Nasr-Esfahani MH1

1. Department of Molecular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Basic Sciences, Najafabad Branch, Islamic Azad University, Isfahan, Iran
Objective: Previous studies confirmed that neural gene expression in embryonic stem cells (ESC) could influence by chemical compounds through stimulating apoptotic pathway. We aimed to use ESCs derived neural cells by embryoid body formation as an in vitro model for determination of neural gene expression changes in groups that treated by sera from Alzheimer’s patients and compare with healthy individuals.

Materials and Methods: ESC line which was derived from the mouse strain was used throughout this study. ESC derived neural cells were treated with serum from Alzheimer’s patient and healthy individual. Neural gene expression was assessed in both groups by quantitative Real-Time Polymerase Chain Reaction analysis. The data was analyzed by SPSS Software (version 18).

Results: Morphologically, the reducing in neurite out growth was observed in neural cells in group, which treated by serum from Alzheimer’s patient, while neurite growth was natural in appearance in control group. Microtubule associated protein 2 and glial fibrillary acidic protein expression significantly reduced in the Alzheimer’s patient group compared with the control group. Nestin expression did not significantly differ among the groups.

Conclusion: Neural gene expression could be reduced in serum treated ESC in Alzheimer’s patients.

Keywords: Neural Cell, Neural Gene Expression, Neurotoxicity

Os-8: A9 Dopaminergic Progenitors Derived from Human Embryonic Stem Cells: Enrichment and Differentiation

Fathi A1, 2, *2, Sharifitabar M1, Salekdeh GhH1, 4, Baharvand H1, 3
1. Department of Molecular Systems Biology at Cell Science Research Centre, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Centre, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Developmental Biology, School of Biology, University of Science and Culture, ACECR, Tehran, Iran
4. Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

Objective: Differentiation of hESCs to Dopaminergic neurons can be achieved in vitro by combination of small molecules during neural ectoderm. Dopaminergic progenitors derived from hESCs are promising tool for the regenerative medicine in Parkinson cell therapy.

Materials and Methods: To enable enrichment and characterization of Mesodiancephalon Dopaminergic (mDA) neural progenitors LMX1A locus of hESC (Royan H6 and Royan H5 cell lines from Royan Institute) targeted with GFP in the upstream of LMX1A coding sequence. GFP protein expressed as fused protein with LMX1A protein and both LMX1aGFP/GFP and LMX1aGFP/w clones were selected for differentiation and fluorescence-activated cell sorting (FACS) to select and purify midbrain dopamine progenitor cells. Initially, the dopaminergic marker profile of NPs cultures was evaluated after differentiation in vitro.

Results: The overlap expression of eGFP with Lmx1a, LMX1b and Corin demonstrated that these cells were of a midbrain dopamine progenitor characters. In addition, enriched dopamine progenitors, which positively selected by FACS were viable, extended neurites, and maintained a dopaminergic profile in vitro, could give raise to mature DA neurons. Engraftment of GFP positive mDA progenitors and NCAM positive NPs as a control to rat model of Parkinson and behavior tests are under studies.

Conclusion: We show that LMX1a could be used as a selection marker of early mDA progenitor’s in vitro and purified mDA will provide invaluable Materials for developmental studies and regenerative medicine.

Keywords: hESC, Neural Differentiation, Dopaminergic Neurons

Os-9: Pioglitazone, A Synthetic Agonist for PPARγ, and b-FGF Enhanced Dissociated Human Embryonic Stem Cells Self-Renewal

Ghaedi K1, 2, *2, Ghoochani A1, Peymani M1, 3, Kajabadi E2, Nasr Esfahani MH1, Baharvand H* 5
1. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Biology, School of Sciences, University of Isfahan, Isfahan, Iran
3. Department of Biology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
4. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Objective: Human embryonic stem cells (hESCs) are pluripotent cells with ability to differentiate to three germ layers. However, dissimilar to mouse embryonic stem cells (mESCs), hESCs are vulnerable to be cultured in dissociated state due to induced apoptosis that has been reflected as an important obstacle to extend genetically manipulation of them. This phenomenon is arisen by activation of Rho/Rock signaling pathway is adversely affecting dissociated hESCs proliferation and survival through E-Cadherin dependent cell-cell adhesion disruption. E-cadherin is one of cytoskeleton elements that will be decreased after Rho/Rock pathway activation. Thus attenuation of Rho/Rock pathway may inhibit apoptosis of dissociated hESCs. One of the proposed components, which have been shown to be potential attenuator of this signaling pathway, is Pioglitzone. Pioglitzone, a specific of agonist of proxisome proliferative activated receptor γ (PPARγ), decreases the MYPT phosphorylation level a
key mediator of Rho/Rock signaling pathway. Proxisome proliferative activated receptor γ (PPARγ) is a member of the PPAR superfamily ligand dependent nuclear receptors (α, β/δ, γ) that has numerous roles in variety of cells. PPARγ could be activated by both natural and synthetic ligands. The aim of this study is implementation of Rock inhibitor (Y-27632) with Pioglitazone to increase hESCs colonies formation rate through induction of E-Cadherin and β-Catenin expression independent of cells survival. Furthermore, we have evaluated the effects of bFGF supplementation on essential transcriptional factors involving in pluripotency of dissociated hESCs and combinational effects of Pioglitazone and bFGF on proliferation state of dissociated hESCs have been examined.

Materials and Methods: To study effects of Pioglitazone on β-Catenin and E-Cadherin along with Rock inhibitor (Y-27632), hESCs were treated with Pioglitazone and Y-27632. Four and 24 hours and 1 week post treating, cells were harvested and quantitative real time PCR was applied to demonstrate effects of using Pioglitazone and Y-27632 on E-cadherin and β-catenin expression. To analyze colony formation rate and cells survival, alkaline phosphatase (AP) and annexinV/ PI tests were performed respectively. Furthermore, hESCs were grown in serum-free N2B27-based media and treated with Pioglitazone in presence or absence of bFGF for 4 days and expression level of pluripotent markers was assessed by real time PCR. Localization and intensity of these markers were also carried out by immunostaining.

Results: Data showed that treating with Rock inhibitor along with Pioglitazone increased hESCs colonies formation rate significantly without influencing cell survival as compared with Rock inhibitor implementation. Moreover, treatment of dissociated hESCs with Y-27632 and Pioglitazone increased E-cadherin and β-catenin expression. Co-treating with bFGF and Pioglitazone increased Nanog expression, whereas did not any significantly effect on OCT4 or SOX2 expression as compared with bFGF supplementation. Moreover, pioglitazone decreased Pax6 and Sox1 as neural precursor cell markers.

Conclusion: The present study suggests that implementation of Rock inhibitor (Y-27632) with Pioglitazone increases hESCs colonies efficiency formation rate through an induction of E-Cadherin and β-catenin expression in an independent pathway of cells survival. FGF/ERK signaling plays a positively acting role in hESCs by supporting Nanog expression. Thus, Pioglitazone could positively effect on NANOG, downstream of FGF2 and decrease PAX6 and SOX1 neural precursor genes, which lead to improvement in self-renewal of hESCs.

Os-10: Induction of Human Cardiomyocyte-Like Cells from Human Fibroblasts Using Recombinant Proteins

Ghazizadeh Z
Department of stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Corresponding Email: xaniar@gmail.com

Objective: Transformation of differentiated cells to induced pluripotent stem cells (iPSCs) has several limitations, including the low efficiency of the process and the necessity of forced expression of at least one pluripotent stem cell transcription factor. Since 2010, different strategies have been utilized in order to achieve induced cardiomyocytes directly from fibroblasts mostly with overexpression of developmentally critical cardiac transcription factors. An alternative approach for reprogramming and direct conversion is transient activation of Oct4, Sox2, Klf4 and c-Myc. Since most of previous studies use retroviral transfection for transcription factor delivery, one of the major issues in application of trans-differentiation in regenerative medicine to be solved is finding a safe tool for this reprogramming process.

Materials and Methods: In the present study, we treated human dermal fibroblasts and human fetal fibroblasts with a combination of 4 proteins (including Oct4, Klf4, Sox2, and c-Myc) conjugated with HIV TAT until fibroblasts enter into an early reprogrammed state. After entering partially reprogrammed state, we directed their differentiation toward cardiac lineage with 20 ng/ml BMP4 treatment for 5 days.

Results: Upon cardiac induction, cells treated with recombinant proteins showed significantly higher expression of early stage and late stage cardiac markers such as GATA4, Mef2C, NKX2.5, αMHC, cTnT and cardiac actin, in comparison to their control. Immunofluorescence staining of transdifferentiated cells showed expressions of NKX2.5, GATA4, CARDIAC ACTIN, GJA, MLC and MHC. We called these cells “induced cardiomyocyte-like cells” (iCLCs). In vivo functionality of reprogrammed cells were tested by injection of differentiated cells to the heart of rats with myocardial infarction. 6 weeks after transplantation heart tissue were analyzed both on macroscopic and microscopic characteristics.

Conclusion: This technique offers safe and fast generation of cardiomyocytes for future applications in regenerative medicine as well as drug screening. Furthermore this novel tool might be a potential method for in vivo conversion of ischemia-triggered aggregation of fibroblasts into cardiomyocytes in infarcted heart.

Keywords: Lineage Conversion, Recombinant Proteins, Cardiomyocytes

Os-11: Differentiation of Hair Follicle Derived Stem Cells to Neural Cells by Induction of Head, Tail, and Limbud Tissue Extract

Khanbabae R, Shojaei S*, Nemati F
Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Qaemshahr, Iran
Corresponding Email: khanbabae@gmail.com

Objective: Adult stem cells are the group of cells which conserve their nature in tissues and organs among other cells. In recent years, the researchers reported the existence of stem cells on the Bulge of hair follicles near to
the smooth muscle. It is possible to differentiate these stem cells to neural cells by induction of Shh, FGF, and RA factors. Because of existence of these factors in head, tail, and limbs of mouse embryo and simplicity and cheapness of achievement to these factors, in this study, we evaluated the differentiation of hair follicular stem cells to neural cells by induction of head, tail, and limbs tissue extract.

Materials and Methods: The adult stem cells isolated from hair follicles of mature mouse (NMRI) and cultured in DMEM/F12 medium which contained EGF. After the first passage in 7th day, these stem cells were induced by head, tail, and limbs tissue extract of 10days mouse embryo whit concentration 50% and 80% during 2 weeks and then the rate of differentiation were assessed.

Results: The immunocytochemical results showed that the expression of Nestin markers was obvious in first week and decreased during 2th week. Moreover, the β tubulin III marker, which is neural cells marker, increased after inducing. The increase of β tubulin III marker in experimental group2 (80%) was significantly more than experimental group1 (50%).

Conclusion: Results of this study showed that the in vitro treatment hair follicular stem cells with tissue extract of 10 days mouse embryo had significant effects on differentiation of hair follicular stem cells to neural cells and the applied concentration of tissue extract was effective on inducing rate.

Keywords: Hair Follicular Stem Cells, Differentiation, Tissue Extract

Os-12: Cell Therapy in Renal Disorders

Moghadasali R

Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Corresponding Email: rezamoghadasali@yahoo.com

A current explanation for development of chronic kidney disease (CKD) is the inequality between damaging mechanism and regenerative repair. At present, dialysis and transplantation remain the only cure options. However, there is trust that stem cells and regenerative medicine may provide additional regenerative options for kidney diseases. Such new treatments might involve induction of repair using endogenous or exogenous stem cells or the reprogramming of the organ to reinitiate development. The possibility that stem cells contribute to the repair of glomerular and tubular injury is of great interest for basic and clinical research. Bone marrow-derived stem cells (BMSCs) have been implicated in the restore of renal tissues. If BMSCs repopulate injured nephrons directly or act indirectly over a paracrine/endoctrine mechanism remains also controversial. Therapeutic administration of BMSCs in animal models of acute kidney injury (AKI) suggests that a stem cell-based therapy may improve the recovery of both glomerular and tubular compartments. Whereas the therapeutic benefit of hematopoietic stem cells (HSCs) remains in doubt, numerous studies showed a beneficial effect of mesenchymal stem cell (MSCs) administration in models of acute tubular injury and of endothelial progenitors in acute glomerular injury. According these studies, our results as an initial report has shown that intra-renal arterial injection of autologous bone marrow mesenchymal stem cells (BM-MSCs) ameliorates cisplatin-induced AKI in a rhesus M. mulatta monkey model. This provides a tool in order to confirm results obtained in murine experimental studies and they are invaluable tools for evaluating cell transplantation protocols prior to their use in clinical studies. Also, recent studies demonstrate the presence of renal stem cells (RSCs) within the adult kidney that responsible for podocytes turnover and potentially also capable to give rise to proximal tubular epithelium cells. These cells are capable, when injected in animals with AKI, to localize to renal compartments and contribute to regeneration. We review the current literature on the role of stem cells in renal regeneration. Moreover, important points that still need explanation, such as the homing mechanisms of stem cells to injured tissues, the secreted factors underlying the paracrine/endoctrine mechanisms and the long-term behavior of in vivo transplanted stem cells, are discussed.

Keywords: Chronic Kidney Disease (CKD), Acute Kidney Injury (AKI), Stem Cells Therapy

Os-13: Co-Administration of Glyburide and Aqueous Extract of Parsley Up-Regulated The Diabetes-Damaged Angiogenesis and GLUT 1 Protein Expression on The Ovaries

Mohamadiraad B1, Razi M1*, Malekinejad H2, Rezaadeh Z1, Mirzakhani N3

1. Department of Comparative Histology Embryology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Pharmacology Toxicology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
3. Department of Pathology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Corresponding Email: mazdak.razi@gmail.com

Objective: Diabetes has been known to decrease follicular growth. Although antihyperglycemic drugs decrease the serum level of glucose, the ovarian tissue impairments as follicular atresia remain unchanged. On the other hand the glucose transporter type I (GLUT 1) expression and ovarian cortical angiogenesis play critical role on theca cells endocrine function and follicular growth, respectively. Thus, present study aimed to evaluate protective effects of glyburide and parsley extract on diabetes-induced damages on the ovary.

Materials and Methods: Thirty Mature female rats were divided into five (N=6) test and control-sham groups. The test group subdivided into four groups. The experimental diabetes induced in all test groups. The test group I (D), test group II (GLY) received glyburide at dose of 5 mg/kg, test group III (P) received parsley extract at the dose of 2g/kg and the simultaneous administration of parsley and glyburide was performed in last test group (P+GLY). After 30 days, the immu-
Objective: Although DMDs reduce the rate of relapses of MS, there is no effective means to stop the progression of disability or induce rebuilding of the destroyed myelin and axons. Adult bone marrow derived stromal cells (MSC) were shown to induce immunomodulatory and neuroregenerative effects and to induce neuroprotection in the animal model of EAE In Vivo. We have designed a randomized clinical trial, semi cross over, Phase I/II study to evaluate the safety and efficacy of intravenous injection of autologous bone marrow derived mesenchymal stem cell in patients with multiple sclerosis.

Materials and Methods: 30 patients (18-55 years, 3<EDSS<6.5) with RRMS, SPMS with relapses or without relapse but we don’t have any judgment because it has not finished yet. 2 patients had relapses and in only one patient 3 severe disabling relapses occurred within 5 months, we did not detect any new GAD+ lesions in the patients MRI.

Conclusion: The Intravenous transplantation of autologous MSC is safe without any adverse effect. Efficacy should be proved at the end of the study.

Key words: MS, Mesenchymal Stem Cell, Safety, Efficacy

Os-15: Generation and Maintenance of Human Embryonic Stem Cells-Derived Cadual Restricted Neural Precursors Cells by Small Molecules

Nemati Sh1*, Shahbazi E1, Karamali F2, Baharvand H3

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Molecular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Corresponding Email: baharvand@royaninstitute.org

Objective: Human embryonic stem cells (hESCs) have led to an important revolution in stem cell research and regenerative medicine. Recent attempts are designed to use them in clinics and its combination with small molecules is another state-of-the-art features of biotechnology for increase our knowledge in stem cell biology. On the other hand, the availability of suitable cell sources for neurodegenerative diseases cell therapy seems negligible. Here, we try to achieve a proper, efficient and well characterize neural progenitor cell line by small molecules.

Materials and Methods: Generating these hESC-NPs was performed by using a combination of small molecules such as SB431542, CHIR99021 and Dorsomorphin after six days of induction. And aslo, we used common methods in cell biology such as Immuno fluorescence staining, flow cytometer and real-time PCR for characterize of these cell lines.

Results: Our results show that the established hESCs-NPs line are closely resemble to real and functional posterior neural progenitors cells.
generate large quantities of enriched stable proliferating NPs using of small molecules. Moreover, it was shown that expandable hESC-NPs were derived in high-density feeder-free hESC cultures without the addition of RA or noggin. Additionally, in terms of the generation of expandable NPs, stability in passaging, and a constant neuro and gliogenic differentiation across many passages, our results are comparable with previously published reports on hESC-NPs.

**Keywords:** Small Molecules, Neural Progenitor Cell, Posterior

**Os-16: Three-Dimensional Pericardium Sponge Improves Proliferation and Differentiation of Sca-1+ Cells**

Rajabi Zeleti S1,2, Jalili Firoozinezhad S2, Azarnia M1, Khayyatian F1, Vahdat S2, Nikeghbalian S1, Baharvand H2*, Aghdamii N2

1. Department of Biology, Kharazmi University, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Shiraz Transplant Center, Namazi Hospital, Shiraz University of Medical Sciences, Iran

**Corresponding Email:** rajabi_s62@yahoo.com

**Objective:** Cardiovascular diseases hold the highest mortality rate among other illnesses which reveals the significance of current limitations in common therapies. So recently, the role of three-dimensional (3D) scaffolds has been highlighted for heart failure post-myocardial infarction treatment. Natural-based scaffolds which resemble more to the damaged tissue of interest seem to be more suitable for necrotic cardiomyocytes replacement and restoring the damaged extracellular matrix (ECM). Here, we have evaluated the properties and potentials of a novel pericardium-derived scaffold for replacing the myocardium ECM.

**Materials and Methods:** Following the decellularization of human pericardium membrane (PM), a 3D sponge pericardium scaffold (PS) was processed with well-defined architecture and interconnected pores. After physico-chemical and mechanical characterizations; MTS assay, histological and migration assessments and Real time PCR were performed in vitro with human Sca-1+ cardiac progenitor cells (CPCs). Thereafter, in vivo assessments of scaffolds were conducted.

**Results:** In comparison with decellularized pericardium membrane (DPM) and collagen sponge (COL), the migration, survival, proliferation and differentiation of CPCs were enhanced on PS. Histological examination of subcutaneous transplanted scaffolds after one month revealed intensive angiogenesis, neovascularization and cardiomyocyte differentiation in PS.

**Conclusion:** Taken together, three-dimensional pericardium sponge may be considered as a good candidate for myocardial tissue engineering applications.

**Keywords:** Pericardium Membrane, Sponge Scaffold, Myocardial Tissue Engineering, Cardiac Progenitor Cells

**Os-17: Engrafted Human Induced Pluripotent Stem Cell-Derived Anterior Specified Neural Progenitors Protect The Rat Crushed Optic Nerve**

Satarian L1,2*, Javan M1, Kiani S2, Hajikarm M2, Mirnajafi-Zadeh J1, Baharvand H2,3*

1. Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

**Corresponding Email:** lsatarian@gmail.com

**Objective:** We examined the survival, integration, differentiation and functional repair potency of human induced pluripotent stem cells-derived neural progenitors (hiPS-NPs) following transplantation into the retina of rats with crushed optic nerve.

**Materials and Methods:** The Royan hiPs were induced to NPs using Noggin and Retinoic acid during 3 week. Q RT-PCR, Immunocytochemistry and flowcytometry were used to detect markers genes and proteins of neural progenitor cells. The expression of ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (bFGF) and Insulin-like growth factor I (IGF1) by hiPS-NPs were detected by ELISA. One hundred rats were randomly divided into four groups: normal control, optic nerve crushed rats injected with PBS, optic nerve crushed rats injected with hiPS-NPs, or injected with dead hiPS-NPs. Cells was labeled by red fluorescent CM-DiI and injected into the vitreous cavity one day after breaking inner limiting membrane by acid aminoadipic acid. Visual evoked potential recording was done for evaluating functional recovery of optic apparatus. Transplanted cells fate was then evaluated using Immunohistofluorescence.

**Results:** hiPS-NPs characterization showed anterior cell characteristics with high expression level of PAX6. ELISA showed neurotrophic factors secretion by hiPS-NPs. Functional analysis using VEPs showed significant amplitude recovery in animals transplanted with live cells. Sixty days after transplantation hiPS-NPs were integrated into the ganglion cell layer of retina and some of them expressed markers of ganglion cells. Retrograde labeling with Dil showed higher number of projection cells in retina.

**Conclusion:** hiPS-NPs transplantation was able to protect retinal ganglion cells after optic nerve injury, and integrated and differentiated in to ganglion cell layer. These cells may provide new therapeutic approach for traumatic optic nerve diseases.

**Keywords:** Human Induced Pluripotent Stem Cell, Neural Progenitors, Optic Nerve Crush, Visual Evoked Potential, Transplantation, Rat

**Os-18: Enhancer DNA Methylation in Differentiated and Stem Cells**

Sharifi Zarchi A
Institute of Biophysics & Biochemistry (IBB), University of Tehran, Tehran, Iran
Corresponding Email: asharifi@gmail.com

Objective: Since the first report about presence of methyl group on some genomic cytosines in 88 years ago there has been a huge effort to decrypt the function of DNA methylation in development, pathogenesis, differentiation and reprogramming. Most of this effort has been focused on specific genomic regions like promoters, CpG islands and gene bodies, and little is known about the role of DNA methylation in other regulatory regions like enhancers.

In this study we aim to focus on DNA methylation at enhancer regions of mouse embryonic stem cells and several semi- or fully differentiated cell types and to make a better insight about interaction between other epigenetic marks including histone modifications, transcription and DNA methylation.

Materials and Methods: Several GEO datasets including whole-genome single nucleotide bisulfite sequencing DNA methylation maps of different cell types, ChIP-seq data of several histone modifications and protein bindings to the mouse genome in different tissues, and RNA-seq transcriptome and non-coding RNA of the same cell types are being used. We have developed an R program for the analysis to check the position-specific pattern of epigenetic and transcriptome marks around enhancers.

Results: We confirm DNA methylation monitors the tissue specific activity of enhancers, in addition to several already identified chromatin marks. Additionally we show new mechanistic interactions between DNA methylation and several histone modifications.

Conclusion: Our results highlight new roles for DNA methylation in leading other epigenetic patterns on enhancers. These findings suggest how some part of epigenome of a cell can be inherited to the offsprings during development and differentiation

Keywords: DNA Methylation, Enhancer, Differentiation, Embryonic Stem Cell, Epigenome, Regulation, Histone Modifications

Os-19: Small Molecule Induction of Human Embryonic Stem Cells Towards Definitive Endoderm

Tahamtani Y1*, Azarnia M2, Farrokh A1, Moradmand A1, Sharifi-Zarchi A1, Aghdami N1, Baharvand H1,3

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
2. Department of Biology, Kharazmi University, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Corresponding Email: yaserta@yahoo.com

Objective: Definitive endoderm (DE) formation is the most important stage that all endodermal organs pass through during their development. So, in vitro production of definitive endoderm is one of the important issues in stem cell related differentiation studies and can help to efficiently produce endoderm derivatives for therapeutic applications. Despite the enormous progress in studying definitive endoderm (DE) differentiation from human embryonic stem cells (hESCs), none of the reported protocols have produced a universal, cost-effective, and competent DE with the capability to further differentiate into endodermal derivatives.

Materials and Methods: In this study, by using a two-step differentiation strategy, we have treated hESCs for one day with “priming” small molecules (SM), [staurprimide, NSC-308848, rapamycin (Rapa), and/or CHIR] and for the next three days with “inducing” SM (LY294002, cymarin, IDE1 and/or IDE2) in conjunction with activin A. In the positive control group, we treated hESCs with Wnt3a (25 ng/ml) for one day and activin A (100 ng/ml; W/A100-A100) for the next three days.

Results: Gene expression analysis showed that treatment of hESCs with 100 nM Rapa and 50 ng/ml activin A (Rapa-A50) out of 25 combinations of factors gave rise to higher expressions of two DE-specific genes, SOX17 and FOXA2. Similar results were obtained after treating two other hESC lines with this regimen. To investigate the competency of Rapa-A50-induced DE for further differentiation into endodermal derivatives, these cells and W/A100-A100-induced DE cells (positive control) were further differentiated into pancreatic progenitors (PP), then into pancreatic endocrine (PE) cells using five previously described differentiation protocols. Gene analysis of differentiated cells showed that the established protocols were insufficient to enable universal differentiation into PE, whereas Rapa-A50-induced DE cells were more competent for PP differentiation in a protocol-dependent manner. Additionally, Rapa-A50-induced DE had the capability to differentiate into hepatocyte-like cells (HLCs) as efficiently as W/A100-A100-induced DE.

Conclusion: These data have indicated that hESCs primed with Rapa, and induced by a lower concentration of activin A, could lead to DE that had the capability to further differentiate into HLCs and PP cells, but not PE cells. Thus, current protocols for the differentiation of DE into PE still need additional study.

Keywords: Definitive Endoderm, Developmental Competency, Human Embryonic Stem Cells, Priming, Rapamycin

Os-20: The Influence of Pioglitazone Administration on The Protection Against Alzheimer's Disease-Induced Apoptosis in Human Umbilical Vein Endothelial Cells

Tahani S1*, Dehghan l1, 2, Meamir R1, 2, Saadatniar M1, Nasr Esfahani MH2

1. Department of Medical Science, Najafabad Branch, Islamic Azad University, Isfahan, Iran
2. Department of Molecular Biotechnology, Cell Science Research Center, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
3. Isfahan Neurosciences Research Center, Isfahan, Iran

Objective: Since the first report about presence of methyl group on some genomic cytosines in 88 years ago there has been a huge effort to decrypt the function of DNA methylation in development, pathogenesis, differentiation and reprogramming. Most of this effort has been focused on specific genomic regions like promoters, CpG islands and gene bodies, and little is known about the role of DNA methylation in other regulatory regions like enhancers.

In this study we aim to focus on DNA methylation at enhancer regions of mouse embryonic stem cells and several semi- or fully differentiated cell types and to make a better insight about interaction between other epigenetic marks including histone modifications, transcription and DNA methylation.

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Conclusion: Our results highlight new roles for DNA methylation in leading other epigenetic patterns on enhancers. These findings suggest how some part of epigenome of a cell can be inherited to the offsprings during development and differentiation

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1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
2. Department of Biology, Kharazmi University, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Corresponding Email: yaserta@yahoo.com

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Os-20: The Influence of Pioglitazone Administration on The Protection Against Alzheimer's Disease-Induced Apoptosis in Human Umbilical Vein Endothelial Cells

Tahani S1*, Dehghan l1, 2, Meamir R1, 2, Saadatniar M1, Nasr Esfahani MH2

1. Department of Medical Science, Najafabad Branch, Islamic Azad University, Isfahan, Iran
2. Department of Molecular Biotechnology, Cell Science Research Center, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran
3. Isfahan Neurosciences Research Center, Isfahan, Iran
**Objective:** Alzheimer's disease (AD) is a progressive neurodegenerative disease which endothelial cell (EC) can be affected by these diseases. Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists (thiazolidinediones, TZDs) such as pioglitazone (PIO) has potent anti-inflammatory property, but increasing evidence suggests that these drugs also improve vascular function and prevent atherosclerotic disease progression.

**Materials and Methods:** Human umbilical vein endothelial cells (HUVECs) were treated with sera from AD patients (n = 20) and sera from controls (n = 10). Apoptosis was identified by annexin V-propidium iodide staining and cell death detection kit. Apoptosis was evaluated after and before adding of 10µM pioglitazone on EC.

**Results:** Induced apoptosis by the serum of patients inhibit markedly when Pioglitazone used before treating HUVECs with the sera of AD.

**Conclusion:** Further studies are justified to investigate the novel role of the PPARs in the prevention of the neuronal and endothelial damage in neurological disorder and present a new therapeutic approach for Alzheimer's patients.

**Keywords:** Apoptosis, Endothelial Cell, Alzheimer's Disease

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**Os-21: Molecular Tracing of mES Cell Derivation from Blastocysts in The Presence of TGF-β and ERK Signaling Inhibitors**

Totonchi M1, 2, 3, Hassani SN1, 2, Tapia N1, Sharifi-Zarchi A1, 4, Marcos J. Araúzo-Bravo5, Greber B5, Sabour D6, Gourabi H7, Hans R. Schöler8, Baharvand H1, 2*

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
3. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
4. Department of Developmental Biology, Max Planck Institute for Molecular Biomedicine, Röntgenstrasse 20, 48149 Münster, Germany
5. Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

**Corresponding Email:** mtotonchi81@yahoo.com

**Objective:** Mouse embryonic stem (ES) cells are derived from inner cell mass of 3.5-day blastocysts and though to exist in a naïve state of pluripotency. During ICM-ES cell course, a normal developmental program is perpetuated as an infinite self-renewal and pluripotency in vitro. In a recent study, we have shown the highly efficient and reproducible generation of ES cells from different murine strains in the presence of TGF-β and ERK signaling inhibitors (R2i).

**Materials and Methods:** To explore the mechanism underlying the acquisition of ground state pluripotency, we performed global gene expression profiling using illumine BeadChip array during the derivation of mouse ES cells.

**Results:** We observed differential patterns of expression for genes involved in gene regulatory network of pluripotency, metabolism, and regulation of cell morphology.

**Conclusion:** This study provides a deeper understanding of mechanisms underlying naïve ES cell derivation and pluripotency regulatory circuitry.

**Keywords:** Naïve Pluripotency Cell, Stem Cell Derivation, mESC

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**Os-22: A Feasible and Efficient Protocol Developed for Scale up Differentiation of Human Pluripotent Stem Cells into Hepatocyte-Like Cells in A Single Suspension Culture**

Vosough M1, 2*, Omidinia E1, Kadivar M1, Shokrgozar MA3, Pournasr B2, Aghdami N3, Baharvand H1, 2, 4

1. Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
4. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

**Corresponding Email:** masvos@yahoo.com

**Objective:** Recent papers described developing protocols to generate hepatocytes from pluripotent stem cells (PSCs) based on simulating developmental stages experimentally. Despite of these wonderful advances, due to inadequate amount of harvested cells their biomedical application is limited. In this regard, considering scale up cultures and practical differentiation protocols are two crucial factors. In this paper, we explain a single suspension culture and differentiation method for manufacturing functional Hepatocyte-Like Cells (HLCs) in stirrer bioreactor.

**Materials and Methods:** Our stepwise protocol composed of serum starvation of cell aggregates, priming with rapamycin (Rapa) and direct induction of them with corresponding growth factors according to natural timetable in developmental biology. In comparison with other protocols in differentiation, this method is feasible, integrated and budget saving, giving the decreased consumption of growth factors.

**Results:** HLCs demonstrated physiological function, e.g. albumin secretion, glycogen storage, urea production, indocyanin green and LDL uptake, collagen synthesis, and CYP activity. These cells were characterized and enriched based on LDL uptake as a biological property and infused intrasplenically to CCL4 treated mice model. Transplanted cells engrafted successfully and increased the survival rate. These cells remained functional after homing and promoted regeneration mechanisms in HLC transplanted group.

**Conclusion:** This practical approach in scaling up and purifying strategy may help scientists to introduce promising methods for cell-based therapeutics and other biomedical application of HLCs.
Ps-1: Comparison of Differential Plating and MACS in Enrichment of Goat Undifferentiated Spermatogonia

Abbasi H1*, Tahmoorespur M1, Hosseini M2, Nasiri Z2, Bahadorani M2, Hajian M2, Nasiri MR1, Nasr Esfahani MH2

1. Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Reproductive Biotechnology at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Corresponding Email: mh.nasr-esfahani@royaninstitute.org

Objective: Spermatogonia are male germ line progenitors of sperm cells. In bovine, rodents and primates, undifferentiated spermatogonia and spermatogonial stem cells (SSCs) express the surface marker THY1. Since cells can be enriched from mixed populations by positive or negative selection on physical or functional characteristics such as cell surface markers, present study was aimed to compare the efficiency of two different enrichment methods: Magnetic activated cell sorting (MACS) and differential plating (DP) in pre-pubertal goat testes cell population. To comparison of two enrichment methods efficiency, real time PCR analysis, protein expression, and transplantation into recipient mice data were analyzed. For control group, the intact testis cells population were used.

Materials and Methods: After 3 times enzymatic digestion of pre-pubertal goat testicular tissues, cells were cultured for 16 h in laminin-coated flasks and DP carried out. For MACS method, cell suspension were sorted using THY1 surface marker.

Results: Real time PCR analysis revealed that expression of THY1, PLZF, VASA, BCL6B, UCHL1 as SSCs characteristic genes in THY1 positive cells were significantly higher than in the DP isolation cells and unselected total cells. Transplantation of THY1 positive, DP isolated and intact population of testis cells (control) into the mice recipient seminiferous tubules revealed that THY1 positive cells had higher capacity for colony formation as compared to DP isolated and intact cells.

Conclusion: The results indicated that isolation of undifferentiated testis cells using MACS method can be more efficient than differential plating.

Keywords: Undifferentiated Spermatogonia, Goat, Testis, MACS, Differential Plating

Ps-2: THY1 As A Reliable Marker for Enrichment of Undifferentiated Spermatogonia in The Goat

Abbasi H1*, Tahmoorespur M1, Hosseini M2, Nasiri Z2, Bahadorani M2, Hajian M2, Nasiri MR1, Nasr Esfahani MH2

1. Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Reproductive Biotechnology at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Corresponding Email: mh.nasr-esfahani@royaninstitute.org

Objective: Spermatogonial stem cells (SSCs) are unique cells of testes that can restore fertility upon transplantation into recipient testes. However, use of suitable markers for enrichment of these cells is an important potential application. THY1 is an established conserved marker of SSCs in bovine, rodents and primates, but, there is no information available in goats.

Materials and Methods: Following three rounds of enzymatic digestion of pre-pubertal goat testicular tissues, undifferentiated spermatogonia positive for THY1 were isolated by magnetic activated cell sorting (MACS) and were used for immunocytochemistry, real time PCR analysis of gene expression, protein expression, and transplantation into recipient mice.

Results: Immunocytochemical analyses showed that significantly higher percentage of THY1+ cells were positive for PLZF and VASA as compared to unselected population. This result for PLZF was further confirmed at protein level. Real time PCR analysis revealed that expression of THY1, PLZF, VASA, BCL6B, UCHL1 as SSCs characteristic genes in THY1+ cells were significantly higher than in the initial population. Finally, transplantation of PKH26 labeled cells revealed that THY1+ cells had higher capacity for colony formation as compared to unselected cells.

Conclusion: The results provide indications that THY1 surface marker can be reliably used for enrichment of undifferentiated spermatogonial in the goats.

Keywords: Undifferentiated Spermatogonia, Goat, Testis, THY1, MACS

Ps-3: Microglial Activation in Rat Experimental Spinal Cord Injury Model

Abdanipour A1*, Tiraahi T2

1. Stem Cells Research Laboratory, Department of Medical Science, Ardabil Branch, Islamic Azad University, Ardabil, Iran
2. Shefa Neurosciences Research Center, Khatam Al-Anbia Hospital, Tehran, Iran

Corresponding Email: abdani.anatomy@yahoo.com

Objective: To understand immune system activation and secondary microglial activation processes after spinal cord injury (SCI).

Materials and Methods: A quantitative histological study was performed to determine ED-1 positive cells, glial cell density and cavitation size in untreated SCI rats at 1 day, 2 days, 4 days, 1 week, 2 weeks, 3 weeks and 4 weeks.

Results: Our results showed that glial cells were the largest population of cells (85.45%), whereas ED-1-immunoreactive cells (monocyte/phagocyte marker in rats) were low (23.15%). Moreover, they infiltrate the injured spinal cord as early as 2 days after the injury.

Conclusion: These findings indicate that multiphase response is observed in contusive SCI. These finding could provide insights into the development of important strategies for treating SCI.

Keywords: Spinal Cord Injury, Inflammation, ED-1 Posis...
Ps-4: Application of Oncolytic Reovirus in Breast Cancer Treatment

Afghah Z, Mashayekhi MH, Nejati Moharrami N*, Niki Boroujeni Z, Aleyasin SA

Department of Medical Biotechnology at National Institute of Genetic Engineering and Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Corresponding Email: nejati.neda@gmail.com

Objective: Cancer is a life-threatening disease that characterized by uncontrolled cell division. Despite modern techniques in diagnosis and therapy, breast cancer is still causing high mortality among women. The use of oncolytic viruses such as Reovirus has received attention recently and currently being used in clinical trials. Reovirus destroys cancer cells with an activated Ras signaling pathway, without damaging healthy human cells.

Materials and Methods: Oncolytic effects of reovirus were explored in a highly malignant and invasive model of breast cancer cells. MTT assay was applied for investigation of optimum dosage of virus for next experiments.

Results: MTT results showed that the optimum dosage of reovirus was obtained as 4.16 μl/30000 cells that has been used in virus infection. Syncytia formation and necrosis were observed 24 hours after infection using optimum virus dosage in cancer cells culture compared to normal cells culture.

Conclusion: These results indicate that reovirus remarkably induce apoptosis in breast cancer cells in vitro and could be a potential tool for targeted cancer therapy in treatment of breast cancer.

Keywords: Breast Cancer, Oncolytic Viruses, MTT Assay

Ps-5: Dose Hyperprolactinemia Induce Reactive Oxygen Species (ROS) Generation In The Testis of Adult Male Mice?

Ahmadi A*, Mostafavi M

Embryology Lab, Department of Basic Science, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Corresponding Email: aram.ahmadi82@gmail.com

Objective: In recent years, the generation of reactive oxygen species (ROS) in the male reproductive tract has become a real concern because of their potential toxic effects at high levels on sperm quality and function. The purpose of this study was to determine and compare the concentration of ROS in testicular homogenate of hyperprolactinemic adult male mice and their effect on sperm motility.

Materials and Methods: 18 adult male mice were categorized in 3 groups as control, sham and treatment. Hyperprolactinemia induction was administrated by 40mg/kg/day Sulpiride atypical antipsychotic drug solution injection for 45days IP. Sham received placebo. After 45days cauda epididymis were removed and placed in HTF medium. After sperm swimming up, the spermatozoal suspension was analyzed for sperm motility. Serum and testicular prolactin were measured in the plasma and testicular homogenate samples of all mice. Induction of oxidative stress ascertained by monitoring the degree of malondialdehyde (MDA) and total nitrite (NO) nm/gr-protein of testicular homogenates.

Results: Higher levels of prolactin plasma concentration and prolactine of tissue homogenate were found in treated group than control and sham groups (p<0.05). Statistically significant decrease in sperm motility was found between treated group and 2 other groups (p<0.05). Additionally MDA and NO levels increased in testicular tissue by hyperprolactinemia. No significant differences were found between 2 other groups.

Conclusion: Hyperprolactinemia by excessive production of ROS causes negative effects on male reproductive function. Oxidative stress-induced damage to sperm may be mediated by lipid peroxidation of the sperm plasma membrane and reduce sperm motility that increases fertility problems.

Keywords: MDA, NO, Hyperprolactinemia, Male Mice, Sperm Motility

Ps-6: Effects of WEE1 Kinase Inhibition on Proliferative Behavior of NSCLC

Ahrari S1, 2*, Jaberipour M3, Razmkhah M3, Hosseini A3, Ahrari I3

1. Department of Biology, School of Science, Shiraz University, Shiraz, Iran
2. Student Research Committee, Shiraz University of Medical Science, Shiraz, Iran
3. Department of Immunology, Institute of Cancer Research, Shiraz University of Medical Science, Shiraz, Iran

Corresponding Email: sajadahrari@yahoo.com

Objective: WEE1 Kinase is associated with controlling the cell cycle progression through G2/M check point. Inhibition of this Kinase has been shown to induce apoptosis in a variety of cancerous cells by skipping the repair mechanisms. In majority of cancer cells, the G1/S check point is damaged due to p53 mutations. So inhibition of this Kinase seems to be a justified approach for induction of DNA damage mediated apoptosis with normal p53 positive cells being excluded. In this study the effect of WEE1 shRNA mediated suppression was evaluated in a cell line from Non small cell lung cancer, considering the reported high profile of WEE1 expression in NSCLC.

Materials and Methods: WEE1 gene was silenced using the specific shRNA in QU-DB cell line. Transfection efficacy was examined by flowcytometry based on GFP expression. 48 hours post-transfection, the fixed DNA content and permealized cells was stained with Propodium Iodide and evaluated by flowcytometry. WEE1 protein expression was also assessed by Western Blotting.

Results: Analysis of Cell Cycle by flowcytometry and after PI staining showed that the majority of treated cells were within the M phase of cell cycle 48h post treatment.
On the other hand, Analysis of protein content in treated cells and the control group by Western blotting was indicative of successful WEE1 suppression.

**Conclusion:** The significant progression through M-phase is suggestive of the prominent role of WEE1 in G2/M regulation. We concerned WEE1 suppression might be a good candidate to fight against NSCLC. Inhibition of cell cycle checkpoint regulators has also been exploited to improve the efficacy of stem cell and iPSCs production. Therefore, this Kinase would be a suggestion for further studies in this field.

**Keywords:** Sh-RNA, WEE1 Kinase, Cell Proliferation

**Ps-7: Epigenetic Alteration of Mir-122 and Let-7b Expression in Adipose Tissue-Derived Mesenchymal Stem Cells by Trichostatin A**

Alizadeh E1*, Zarghami N1, Eslaminejad MB2, Barzegar A3, Jahangir S2, Hashemzadeh S4

1. Department of Medical Biotechnology, School of Advanced Medical Science, Tabriz University of Medical Science, Tabriz, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, , Tehran, Iran
3. Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Tabriz, Iran
4. Department of General Surgery, Faculty of Medicine, Tabriz University of Medical Science, Tabriz, Iran

**Corresponding Email:** zarghami@tbzmed.ac.ir

**Objective:** Adipose tissue derived-mesenchymal stem cells (AMSCs) have the potential of differentiation into different lineages. Abundance and non-invasive isolation of AMSCs made them favorite choice for autologous stem cell therapy. MicroRNAs are small, non-coding RNAs with great impact on proliferation and differentiation. Recent study pointed that let-7-family are main micro-RNAs in exosomes deriving from AMSCs. Also, miR-122 is liver-specific microRNA. Histone-deacetylase inhibitors such as Trichostatin A are epigenetic agents with great impact on proliferation and differentiation. The aim of this study was to investigate the effect of Trichostatin A (TSA) on expression levels of mir-122, let-7a, let-7b, let-7c and let-7d in AMSCs.

**Materials and Methods:** Subcutaneous adipose-tissue was obtained with informed consent during surgery from 6 donors in Teaching-Imam Reza Hospital of Tabriz, Iran. Isolation of AMSCs was performed using enzyme digestion and established-protocol. AMSCs were stained with combinations of antibodies conjugated with FITC or PE: CD34, CD44, CD45, CD11b, CD45, and CD90 followed by flow cytometry analysis. Differentiation potential of AMSCs was evaluated by osteogenic and adipogenic induction. AMSCs were cultured in media containing L-DMEM, EGF, ITS, OMS, and bFGF, and various concentrations (0-25μM) of TSA. The colony-forming and MTT assays were performed. The expression of mir-122, let-7a, let-7b, let-7c, and let-7d was investigated by LNA-based Real time PCR in AMSCs, at days 7th, 14th, and 21st after TSA treatments.

**Results:** Our isolated AMSCs expressed CD44, CD73, and CD90 markers (95-97%). Epithelial-like morphology was observed in AMSCs, surrounded by fibroblastic-cells 20 days after culturing with TSA. Additionally, among the human miRNAs investigated by the real time PCR, miR-122 was induced and conversely let-7b miRNAs was down-regulated in the TSA-treated group as compared to the control group.

**Conclusion:** These results imply that mir-122 and let-7b might have a possible role in differentiation process. So, these findings may be applicable in production of functional hepatocytes from AMSCs utilizing microRNAs and epigenetic agents.

**Keywords:** Mir-122, Let-7b, Expression, Adipose Tissue-Derived Mesenchymal Stem Cells, Trichostatin A

**Ps-8: The Effect of Shear Stress on Differentiation of Human Adipose-Derived Mesenchymal Stem Cells into Endothelial Cells by A Perfusion Bioreactor**

Amirzadeh M1, 2, 3, Amoabediny G1, 3, 4, Haghighipour N2, 3, 4, Yazdian F1, 2, Ghazizadeh L3

1. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
2. Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
3. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
4. Department of Chemical Engineering, Faculty of Engineering, University of Tehran, Tehran, Iran

**Corresponding Email:** mahan.amirzade@gmail.com

**Objective:** Vascular endothelial cells (ECs) are subject to hemodynamic stimuli such as fluid shear stress (SS) in vivo. SS is the most important mechanical factor which affects ECs; also SS induces differentiation mesenchymal stem cells (MSCs) into ECs. In the present study, the effects of SS on endothelial differentiation of human adipose-derived mesenchymal stem cells (hASCs) utilizing a bioreactor with different rates of SS is investigated by evaluation of vWF expression.

**Materials and Methods:** The stem cells were studied in separate groups: control, chemical and mechanical groups. After isolation of hASCs from abdominal fat tissue of healthy women between 25-34 years old, characterization by flowcytometry and assessing multilineage differentiation potential, at passage 3 cells were used in experimental groups. In chemical test, MSCs treated by collagen type1 coated silicon tube and further exposed to SS in three different amplitudes (0.6-1.06- 1.5 dyn/cm²) for 24 hours in bioreactor. hASCs without any specific treatment in normal culture conditions were considered as negative control and Human
Ps-9: Differentiation Capacity of Mesenchymal Stem Cells and Their Potential to Generate Neuronal and Glial Lineage

Azedi Tehrani F1*, Kazemnejad Leili S2, Zarnani AH3, Behzadi G1, Khanmohammadi M2, Khajani S1, Talebi S1, Edalatkhah H4, Akhondi MM5

1. Department of Physiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Embryology and Stem cells at Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Department of Nanotechnology at Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
4. Department of Genetics at Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran

Corresponding Email: S.kazemnejad@avicenna.ac.ir

Objective: Human menstrual blood is easily accessible, renewable, and inexpensive source of stem cells. There is a little information about neural differentiation capacity of menstrual blood derived stem cells (MenSCs). In order to examine feasibility of MenSCs for neural tissue engineering and regenerative medicine, differentiation potential of MenSCs into neural- and glial-lineages was compared with bone marrow mesenchymal stem cells (BMSCs) as well-known stem cell source.

Materials and Methods: Menstrual blood samples were collected from 5 healthy females using Divacup. Bone marrow aspirates were also obtained from iliac crests of human donors that filled the informed consent. After characterization of MenSCs and BMSCs using flow cytometry, their differentiation into neuronal- and glial-like cells was investigated in vitro. The parallel experiments were carried out to characterize neural markers such as Nestin, GFAP and beta-tubulin III using immunofluorescence staining and Real-time PCR in differentiated cells.

Results: Based on flow cytometric analysis, the isolated MenSCs exhibited typical expression of mesenchymal stem cell markers similar to that of BMSCs. However, marked expression of OCT4 was obtained only in MenSCs. The fibroblastic morphology of both MenSCs and BMSCs was turned into neurosphere-like shape during first step of differentiation. The morphological change of both MenSCs and BMSCs during different steps of differentiation was accompanied with elevating expression of Nestin and GFAP protein. Although the mRNA level of Nestin and beta-tubulin III in differentiated MenSCs was higher than that in differentiated BMSCs (p<0.001), the up-regulation level of these markers was lower in differentiated MenSCs compared to BMSCs.

Conclusion: Our results demonstrated that MenSCs are unique stem cell population with differentiation ability into neuronal- or glial-like cells with a different pattern compared to BMSCs.

Keywords: Menstrual Blood, Bone Marrow, Mesenchymal Stem Cell, Differentiation, Neuron

Ps-10: The Effect of Bisphenol A on Osteogenic Activity and Morphology of Rat Bone Marrow Mesenchymal Stem Cells In Vitro

Azimi AS1*, Lofti B, Soleimani Mehranjani M, Mahdiyeh M, Shariatzadeh SMA, Shojafar E, Khandipour A

Department of Biology, Faculty of Science, Arak University, Arak, Iran

Corresponding Email: atenazimy@yahoo.com

Objective: Bisphenol A known to be a xenoestrogen, is widely used in industry and dentistry. In addition to environmental problems, also can be harmful to human health. This study was aimed to evaluate the effect of bisphenol A (BPA) on the viability and osteogenic differentiation in rat bone marrow mesenchymal stem cells to osteoblast.

Materials and Methods: Rat bone marrow mesenchymal stem cells were extracted under sterile conditions using flushing-out method. At the end of third passage, cells were divided to 2 groups control and bisphenol A (250nM), for a period of 21 days in the osteogenic media (DMEM containing 10% fetal bovine serum, 10 mM β-glycerol phosphate, 10 nM dexamethasone and 50 g/ml ascorbic 3-phosphate). The cell proliferation and viability, bone matrix mineralization, intercellular calcium deposition, extracellular calcium deposition, alkaline phosphatase activity and morphologic changes of the cells were measured using MTT assay, alizarin red staining, calcium kit, von kossa staining, alkaline phosphatase kit and Fluorescence staining respectively. Data was analyzed using way ANOVA and means difference was considered significant at p<0.05.

Results: A significant reduction in the cell viability, mineralization and calcium deposition, alkaline phosphatase activity, change of chromatin condensation, and cytoplasm morphology was seen in the cells treated with bisphenol A, whereas These parameters were significantly increased in the control level.

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Ps-11: The Effect of α-Tocopherol on The Osteogenic Differentiation and Morphologic Changes in Rat Bone Marrow Mesenchymal Stem Cells In Vitro

Azimi AS*, Soleimani Mehranjani M, Mahdiyeh Najafabadi M, Shariatzadeh SMA

Department of Biology, Faculty of Science, Arak University, Arak, Iran
Corresponding Email: atenazzim@yahoo.com

Objective: α-tocopherol as a strong antioxidant plays an important role in scavenging of free radicals. α-tocopherol has been found to have antioxidant and cytoprotective properties in cultured cells but its effect on osteogenic differentiation in bone marrow mesenchymal stem cells has not yet been determined.

Materials and Methods: Rat bone marrow mesenchymal stem cells were extracted under sterile conditions using flushing-out method. At the end of third passage, cells were divided to 2 groups control and α-tocopherol (15, 25 µM), for a period of 21 days in the osteogenic media (DMEM containing 10% fetal bovine serum, 10 mM β-glycerol phosphate, 10 nM dexamethasone and 50 g/ml ascorbic 3-phosphate). The cell proliferation and viability, bone matrix mineralization, intercellular calcium deposition, extracellular calcium deposition, alkaline phosphatase activity and morphologic changes of the cells were measured using MTT assay, alizarin red staining, calcium deposition, extracellular calcium deposition, alkaline phosphatase activity, and Fluorescence staining respectively. Data was analyzed using way ANOVA and means difference was considered significant at p<0.05.

Results: A significant rise in the cell viability, mineralization and calcium deposition, alkaline phosphatase activity, change of chromatin condensation, and cytoplasm morphology was seen in the cells treated with α-tocopherol.

Conclusion: Since α-tocopherol caused a significant increase in viability, osteogenic differentiation in the rat bone marrow mesenchymal stem cells, therefore it can be used to treat osteoporosis.

Keywords: Mesenchymal Stem Cells, α-tocopherol, Cell Viability, Osteogenic Differentiation

Ps-12: Effects of Amniotic Membrane Extract on Umbilical Cord Blood Mesenchymal Stem Cell Expansion

Babaei A*, Vojdani Z, Vasaghi A, Habibagahi M, Talaei T

Department of Stem Cells and Developmental Biology at Cell Science Research Center, School of Medicine, Shiraz University, Shiraz, Iran

Objective: Amniotic cord blood is a good source of mesenchymal stem cell that can be expanded and stored in the cell bank and used in regenerative medicine. The objective of this study was to test whether amniotic membrane extract (AME), as a rich source of growth factors such as basic-fibroblast growth factor (bFGF), can promote the potential of cellular proliferation in UCBMSC. The proliferation rate of the growing cells was measured by BrdU assay. Duplication number and time were calculated. Cultured cells were assessed for expression of stem cell’s specific markers by flowcytometry. The differentiation capacity of the UCBMSC toward osteogenic and adipogenic lineages was also evaluated.

Materials and Methods: UCBMSCs were cultured in the presence of bFGF and AME and compared with those cells cultured in media without supplementation. The proliferation rate of the growing cells was measured by BrdU assay. Duplication number and time were calculated. Cultured cells were assessed for expression of stem cell’s specific markers by flowcytometry. The differentiation capacity of the UCBMSC toward osteogenic and adipogenic lineages was also evaluated.

Results: BrdU assay showed a significant increase in the proliferation rate of AME -treated cultures. Similarly supplementation of the culture media with AME and bFGF led to a significant increase in the duplication number and a decrease in the duplication time without any change in the cell morphology. Both AME and bFGF altered the expression of CD44 and CD105 in UCBMSC population. Adding bFGF but not AME to the culture media favored the differentiation potential of UCBMSC toward osteogenic lineage.

Conclusion: AME may enhance the proliferation rate and duplication number of the stem cell through changing the duplication time.

Keywords: Amniotic Membrane Extract, Basic Fibroblast Growth Factor, Umbilical Cord Blood Mesenchymal Stem Cell

Ps-13: Histopathological Evaluation of Adipose-Derived Mesenchymal Stem Cell on Cartilage Defects of Knee in Male Rabbits

Babazadeh M*, Mehrabani D2, Tanideh N1, 3, Ashraf MJ4, Hoseinzadeh S3, Tamaddon A5, Heidari M6, Kuhi O7, Parvin F2

1. Department of Veterinary Medicine, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
2. Stem Cell and Transgenic Technology Research Center, School of Medicine, Shiraz Medical University, Shiraz, Iran
3. Department of Pharmacology, School of Medicine, Shiraz Medical University, Shiraz, Iran
4. Department of Pathology, Khalili Hospital, Shiraz Medical University, Shiraz, Iran
5. Department of Public Health and Food Hygiene, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
6. Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
7. Animal Laboratory, School of Medicine, Shiraz Medical University, Shiraz, Iran
Corresponding Email: vet.marziyeh@gmail.com

Objective: Partial-thickness defects evolving in mature articular cartilage do not heal spontaneously. Tissue en-
烃工程 has long been investigated to repair articular cartilage defects. The current study was designed to observe chondrogenic differentiation of adipose derived stem cells (ASCs) to repair partial-thickness articular cartilage defects in non-weight bearing area in adult rabbit.

Materials and Methods: A partial-thickness (without penetration of the subchondral bone, with 4 mm in diameter) cartilage defect was created in the medial femoral condyle of twelve adult Dutch rabbits. Autologous mesenchymal stem cells isolated from subcutaneous adipose tissue of the same rabbit harvested were injected intra-articularly after the creation of the defect. Defects without treatment were used as controls. The rabbits were sacrificed at 4 and 8 weeks for histological analysis. Healing of the defect was investigated histologically using haematoxylin and cosin, safranin-O staining and toluidine blue.

Results: The results showed that in treatment groups, at 8 weeks post-surgery, the defects were resurfaced with hyaline-like tissue and an ideal interface between the engineered cartilage, the adjacent normal cartilage, and the underlying bone was observed. In contrast, at 4 weeks post-implantation was partially filled with repair tissue, but only half of the repair tissue was hyaline cartilage. Defects were only filled with fibrotic tissue in controls. The findings showed significant differences in the quality of cartilage between ADSCs-injected groups compared to control group, particularly at 8 weeks after surgery

Conclusion: Stem cells that exist in the adipose tissues are able to renew themselves through cell division without changing their phenotype and are able to differentiate into chondrogenic lineage under certain physiological or experimental conditions. These findings suggest that it is feasible to repair articular cartilage defects and maintain long-term viability with no evidence of tumorigenicity, providing a safe, highly-efficient and practical strategy with implants generated by seeding autologous ADSCs, without in vitro differentiation for cartilage tissue engineering.

Keywords: Adipose Derived Stem Cells, Cartilage Defect, Histopathology

Ps-14: MRI Evaluation for Repairing Effects of Adipose-Derived Stem Cells on Cartilage Defects of Rabbit Knee Joints

Babazadeh M1*, Tanideh N2,3, Mehrabani D2, Lotfi M4, Ahrari S5, Hoseizadeh S6, Tamaddon A2,5, Heidari M5, Kuhi O2, Parvin F2

1. Department of Veterinary Medicine, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
2. Department of Stem Cell, Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Medical Imaging Research Center, Department of Radiology, Shiraz University of Medical Sciences, Shiraz, Iran
5. Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Objective: There are limited treatment options for cartilage defects in clinical practice because of the lack of suitable bioMateri. Adipose stem cells (ASCs) are believed to be multipotent in differentiation into chondrogenic lineage. This study assessed the effectiveness of intra-articular injection of autologous ASCs to repair a full-thickness articular cartilage defect in the medial femoral condyle of rabbits to augment healing of defects.

Materials and Methods: A full-thickness (without penetration of the subchondral bone, with 4 mm in diameter) cartilage defect was created in the medial femoral condyle of twelve adult Dutch rabbits. Allogeneic ASCs harvested from subcutaneous adipose tissue were injected intra-articularly after the creation of the defect. Defects without treatment were used as controls. The rabbits were sacrificed 4 and 8 weeks after surgery for MRI analysis using BLOKS scoring system.

Results: The rabbit knee joints were underwent dynamic changes during the process of tissue repair in the defective area after operation. No statistically significant differences were detected between BLOKS scoring system characteristics of all the groups (p>0.05). Control groups showed a deal amount of intra-articular effusion with obvious peripheral tissue edema and a majority of bone marrow lesions. However, these characteristics were not present in treatment groups.

Conclusion: The results showed that injection of ASCs prevent progression of lesions in treatment group whereas there were no repairing effects of ASCs in control group.

Keywords: Adipose-Derived Stem Cells, Cartilage Defect, MRI

Ps-15: An Efficient Method for Isolation and Identification of Ovine Fetus Bone Marrow Mesenchymal Stem Cells

Bagheri F5*, Daliri Joupari M4, Lotfi M5, Babazadeh M1, Lotfi M4, Ahrari S5, Hoseizadeh S6, Tamaddon A2,5, Heidari M5, Kuhi O2, Parvin F2, Lotfi M4, Ahrari S5, Hoseizadeh S6, Tamaddon A2,5, Heidari M5, Kuhi O2, Parvin F2

Objective: Mesenchymal stem cells (MSCs) are vastly used in different disease cell therapies, like cardiovascular disease. Most research is done on small model animals particularly mice. However significant differences exist between small animals and human. For this reason, we developed an efficient method of MSC isolation from ovine fetus which does not have the aging problems of adult MSCs.

Materials and Methods: Bone marrow aspirations were isolated from different bones like humerus, femur and tibia separately. MSCs were isolated in Ficol gradient from bone marrow aspirations and cultured in...
DMEM medium. The forth passage-cells were analyzed with CD44 and CD34 markers to identify MSCs by Flow cytometry. Also we used CHEMICON’s Alkaline Phosphatase Detection Kit.

**Results:** Flow cytometric analysis revealed that the cells expressed high levels of human matrix markers. They were positive for CD44 and negative for CD34. We applied alkaline phosphatase to detect stem cells and observed that colonies expressed alkaline phosphatase. In femur and humorous samples, there were cells identified as monocytes by IHC. They omitted MSCs from culture medium, so to separate them from each other, we used gelatin coated plates.

**Conclusion:** To identify ovine MSCs, we can use anti human MSC markers instead of anti ovine MSC markers. Also to get more homogenous MSCs without monocytes, it is better to use tibia bone marrow. We suggest that using gelatinated plates is the optimal condition for separating MSCs and monocytes.

**Keywords:** Ovine, Mesenchymal Stem Cells, Flow cytometry, Bone Marrow

**Ps-16: Propagation of Caprine Spermatogonial Colonies by Different Growth Factors**


1. Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran
2. Department of Reproductive Biotechnology at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Embryology at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

**Objective:** Spermatogenesis depends on spermatogonial stem cell self renewal and differentiation, but these cells are very few in each testis approximately 0.02-0.03% in mouse testis. In the present study, cell lines from neonate caprine testes were cultured and characterized and the effect of growth factors were investigated. Monitoring cultured cells after 4 and 7 days revealed colonies resembling spermatogonial colonies contained spermatogonial stem cells (SSCs).

**Materials and Methods:** Growth of these colonies depended on the growth factors: leukemia inhibitory factor (LIF), epidermal growth factor (EGF), glial-cell derived neurotrophic factor (GDNF), GDNF receptor α-1 (GFR-α-1), Insulin like growth factor-I (IGF-I) and basal fibroblast growth factor (bFGF). Basal media (BM), BM plus GDNF and BM with GDNF and GFR-α-1 had the most number of colonies in compare with BM contains GDNF, IGF-I and bFGF, BM with GDNF, EGF and LIF and BM plus all growth factors in day 4.

**Results:** However seven days after culture, the least and insignificant decrement in number of colonies observed in media supplemented with IGF-I and bFGF. In day 4 the least number of colonies were achieved in effect of GDNF, EGF and LIF. In the other hand, these growth factors had putative effects on proliferation of somatic cells. The basal media was supplemented with all growth factors provided good conditions for both somatic cells and spermatogonial colonies. Furthermore we determined spermatogonial colonies by immunocytochemistry with SSC, germ cell and somatic cell markers.

**Conclusion:** Characterization of these colonies revealed that all aforementioned markers were positive in all colonies of experimental media. RT-PCR analysis of SSC markers and transplantation of colony cells confirmed that media containing GDNF+IGF-I+bFGF was the best mixture for caprine SSC maintenance.

**Keywords:** Caprine, Spermatogonial Stem Cells, Culture, Growth Factors

**Ps-17: Neural Differentiation of Adipose Tissue-Derived Stem Cells Is Improved Following Coculture with Embryonic Stem Cells**

Bahmani L*, Javeri A, Fakhrtaha M

Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

**Corresponding Email:** leila.bahmani6508@yahoo.com

**Objective:** The aim of the present study was to evaluate neural differentiation of adipose tissue-derived stem cells (ADSCs) following coculture with embryonic stem (ES) cells.

**Materials and Methods:** ADSCs were isolated from the inguinal adipose tissue of mice. Third-passaged ADSCs were cultured to reach 90% confluency. Neural differentiation of ADSCs was performed in DMEM supplemented with Knockout Serum Replacement (KoSR). RoyanB1 ES cells were seeded into Transwell-Clear inserts of 6-well tissue culture plates and cocultured with third-passaged ADSCs for two days. Culture inserts were then removed and neural differentiation was initiated in DMEM supplemented with KoSR. Two weeks after the initiation of differentiation, the expression of neural-specific markers in the differentiated ADSCs was assessed by RT-PCR, quantitative real-time PCR and immunocytochemistry.

**Results:** 24 hours after the initiation of differentiation, ADSCs showed a neuron-like morphology. Coculture of ADSCs and ES cells improved neural differentiation of the ADSCs. Differented ADSCs showed the expression of PAX-6, Nestin, NSE, NeuN, NEFL, TH and ChAT mRNAs. Neuron-specific proteins like MAP2, NEFL and β-tubulin III were also detected in the differentiated ADSCs by immunocytochemistry. Based on quantitative real-time PCR, the expression of neural specific genes increased after Coculture of ADSCs with ES cells.

**Conclusion:** ADSCs exhibited an excellent potential for differentiation toward neurons, and neural differentiation
Ps-18: Serial Sphere Formation, A Method for Enrichment of Gastric Cancer Stem Cells; An Experience on AGS Cell Line

Bakhshi M*, Ebrahimi M2, Asadi J1, Hajimoradi M3

1. Department of Molecular Medicine, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
2. Department of Stem Cells and Developmental Biology at Haematopoetic and Cancer Stem Cell Research Center, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran
3. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Corresponding Email: marzieh_99@yahoo.com

Objective: Gastric cancer is one of the most principal causes of cancer-related death worldwide. To overcome therapeutic challenges, new investigations about cancer pathogenesis and tumor relaps have been tended to “cancer stem cells” (CSCs) as previously neglected targets. Identification and characterization of CSCs could help to develop novel therapeutic strategies in gastric cancer. Since the specific CSC markers have not been idefined in many of cancers, recently, functional approaches including spheroid body formation have been taken into consideration for enrichment of the CSCs.

Materials and Methods: Tumour cells from adenocarcinoma cell line "AGS" were cultured in RPMI 1640. In order to enrich the gastric cancer stem cells, cultured cells transfered to low attached plates and cultured in serum-free medium supplemented with growth factors: EGF, b-FGF and B27 to form spheroids. Serial suspension cultures were done by passaging sphere derived cells into the new plates at the same condition each 8 days. After passage 4, cells were removed to be characterized by colonogenic assay, drug resistance to cisplatin and docetaxel by MTS assay, marker expression evaluation for putative stemness-markers: CD44,CD133,CD24 and CD326 (EPCAM) at the protein level using flow cytometry and sox2, oct4, klf-4, nanog and c-myc at the level of mRNA.

Results: Clonogenicity of subsphere-derived cells was significantly higher than parental cells (p<0.05). The screened cells showed more chemoresistance to cisplatin and docetaxel after 72 hours exposure Compared with non selected cells (p<0.05). In addition the stemness genes were up regulated in this population; indicating that their undifferentiated state was retained. Flowcytometric MFI index of Stemness-markers: CD44 and EPCAM, was also higher in sphere-derived group than control (parental tumour cells).

Conclusion: Our results demonstrated that serial sphere formation assay could be a beneficial model for enrichment of cancer stem-like cells. However it needs to be confirmed by in vivo tumorigenicity models.

Keywords: Gastric Cancer, Cancer Stem Cell, Sphere, Chemoresistance, Stemness

Ps-19: The Programmed Cell Monocyte Origin

Beikzadeh B*, Delirezeh N

Department of Microbiology, Veterinary Medicine Faculty, Urmia University, Urmia, Iran

Corresponding Email: bbeikzadeh@live.com

Objective: The ultimate goal of regenerative medicine is to replace lost or damaged cells. Humans have a limited capacity to regenerate and restore their tissues and organs (such as blood and the liver). The use of stem cells to replace or repair damaged cells and tissues is considered, but the plasticity of adult stem cells is controversial and on the other hand these cells are a rare population with restricted potential to differentiate. Also the use of these cells is limited because paucity of specific markers, difficult propagation in culture and requiring a long period of the time until sufficient cells are obtained. Recent studies show that peripheral blood monocytes have stem cell-like features. We evaluated the potential of dedifferentiation and proliferation capacity of human peripheral blood monocytes in the presence of IL-3 and M-CSF to convert into programmed cell monocyte origin (PCMO).

Materials and Methods: Peripheral blood mononuclear cells (PBMC) were isolated by using Ficoll/Hypaque. Then monocyte isolated by plastic adherence technique. The Monocytes were cultured with M-CSF and IL-3 for 6 days. Then morphology, phenotype and proliferation of PCMOs by Light microscopy, Immunocytochemstry-Flowcytometry and MTT assay respectively were analyzed.

Results: We found that the monocyte in the presence of IL-3 and M-CSF became confluent, which was the result of both an increase in cell size and proliferation. Phenotype analyses, performed at 6th, indicated that CD45, CD34, CD177, CD15 were up-regulated after 6 days and other markers (CD68, CD1a, CD14) remained low.

Conclusion: We conclude that the monocyte dedifferentiated, proliferated and become reprogrammable in the presence of IL-3 and M-CSF. These cells can be re differentiated or even switch to another cell type for use in regenerative medicine.

Keywords: Monocyte, Stem Cell, Dedifferentiated, PCMO

Ps-20: Mesenchymal Stem Cells, Dendritic Cells and Their Role in Organ Transplantation

Beikzadeh B*, Habilbian R

Department of Microbiology, Veterinary Medicine Faculty, Urmia University, Urmia, Iran

Corresponding Email: bbeikzadeh@live.com

Objective: Transplantation is the only treatment choice for many patients with organ failure. Currently, graft rejection is controlled by immunosuppressive drugs, which has the broad side effect. Hence, therapeutic strategies to
reduced dependence on immunosuppressive drug therapy and the induction of donor-specific tolerance. New insights into stem cell biology raise the possibility of using stem cells to modulate the immune response and induce tolerance in organ transplantation.

**Materials and Methods:** Dendritic cells (DCs) are a central player in all immune responses that specialized to initiate graft rejection. These cells are capable of activating T lymphocytes, in both donor and recipient can act as a foe and friend. Mesenchymal stem cells (MSCs) are a heterogeneous, non-haematopoietic, multipotent, adult stem cells that are isolated from different sources. This features makes it easier to access these cells than the other stem cells. Studies have shown that MSC can impaired the differentiation of monocytes or CD34+ haematopoietic stem cells into DCs by inhibiting the response of to maturation signals, reducing the expression of costimulatory molecules and hampering the ability of the former to stimulate naïve T cell proliferation and IL-12 secretion. This function is mediated by a non-specific anti-proliferative action, which is dependent on cell-cell contact or secreted soluble factors.

**Results:** Following this interaction between MSC and DC, immunomodulation MSC mechanism causes the modulation of CD8+ cells towards a non-cytotoxic/suppressor cells and induction of CD4+CD25+FoxP3+ cells.

**Conclusion:** Generally, allogeneic MSCs may potentially induce a state of tolerance by modulating the generation, activation, and function of DCs, which it should be beneficial in graft survival especially in inhibition of graft versus host disease.

**Keywords:** Mesenchymal Stem Cells, Dendritic Cells, Transplantation, Immunomodulation

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**Ps-21: miR-22 As A Stem Cell Specific MicroRNA Reveals Differential Expression in Tumoral and Non-Tumoral Breast Tissue**

Damavandi Z1*, Vasei M2, Tavallaee M3, Mowla SJ1

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran  
2. Pathology Laboratory, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran  
3. Human Genetic Research Center, Baqiyatallah Hospital, Baqiyatallah University of Medical Sciences, Tehran, Iran  
Corresponding Email: sjmowla@modares.ac.ir

**Objective:** MicroRNAs (miRNAs) are a major class of small endogenous RNA molecules that post-transcriptionally inhibit gene expression. Many miRNAs have been implicated in several human cancers, including breast cancer. Breast cancer is the most frequent form of cancer in women and Cancer stem cells (CSCs), or cancer cells with stem cell properties, have been reported in many human tumors and are thought to be responsible for tumor initiation, therapy resistance, progression, relapse, and metastasis. The cancer stem cell hypothesis postulates that tumors are maintained by a self-renewing CSC population. MiRNAs play critical roles in normal stem cell functions during development, have emerged as important regulators of CSCs as well. Several miRNAs, including miR-22, are highly expressed in mammary progenitor cells, while others, including let-7, are depleted so miR-22 can act as a self-renewal microRNA in breast cancer stem cell (BCSC) which is located in 17p13.3 genomic locus. The aim of this study is to evaluate the expression alteration of miR-22 as a BCSC specific marker.

**Materials and Methods:** A matched case-control study was conducted that included tumor and matched non-tumor surgical specimens from patients diagnosed with breast invasive ductal carcinoma. Formalin-fixed, paraffin-embedded (FFPE) samples were prepared for RNA extraction with using xylene-ethanol method then Total RNA was isolated using TRizol reagent. Specific stem-loop primer was designed and cDNA synthesized particularly. The expression of MicroRNA was evaluated by Real-time PCR.

**Results:** According to our data, miR-22 expression revealed alternation in tumor versus non-tumor samples of breast tissue. Also indicated our stem-loop primer synthesized cDNA specifically.

**Conclusion:** Despite the alternation expression of miR-22 in breast tumor could claim the presence of CSCs in tumor cell population. However, introducing miR-22 as a discrimination factor of tumor state is under study and needs further investigation.

**Keywords:** miR-22, Cancer Stem Cell, Breast Cancer

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**Ps-22: Synergistic Inhibitory Effects of Tamoxifen and Tranilast on VEGF Expression and Secretion in Cultured Human Breast Cancer Cells**

Darakhshan S1, Ghanbargi A1*, Khazaei M2

1. Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran  
2. Department of Anatomy, Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran  
Corresponding Email: aligharak@yahoo.com

**Objective:** Worldwide, breast cancer is a major cancer leading in both incidence and mortality in women. Angiogenesis plays essential role in breast cancer growth and progression, also a major requirement for breast tumors to successfully spread. VEGF is a main player in the neoangiogenesis of neoplastic breast tissues. Tamoxifen is a synthetic non-steroidal anti-estrogenic drug widely used for the treatment of breast cancer. Tranilast is an anti-allergic agent used mainly to treat allergic diseases. The aim of present study was to examine the effects of anti-cancer tamoxifen and anti-allergic tranilast as a single or in combination on the proliferation and VEGF level in breast cancer cells in vitro.

**Materials and Methods:** The human breast cancer cell line, MCF-7 was treated with graduated concentrations of tamoxifen and/or tranilast. Cytotoxic effects of tamoxifen and tranilast alone or in combination on percent cell...
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Ps-23: Embryonic Stem Cell in Drug Toxicity: Genetically Engineered Versus Non-Genetically Engineered Cells in Cardiotoxicity Model

Dehghani L1,2*, Farokhpour M1, Tahani S2, Nasr Esfahani MH1, Baharvand H3

1. Department of Molecular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Medical Sciences, Najafabad Branch, Islamic Azad University, Isfahan, Iran
3. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Corresponding Email: L_dehghani2002@yahoo.com

Objective: Embryonic stem cells (ESCs) have various uses in drug toxicity, as they can be easily differentiated in vitro. However, one of the major obstacles in the assessment of these differentiated cells is the presence of a heterogeneous cell population.

Materials and Methods: To circumvent this problem, purified ESC-derived desired cells by means of the tissue-specific GFP and/or antibiotic resistance gene expression has been proposed. Therefore, this study aimed to assess the role of doxorubicin (DOX) in cardiotoxicity by using genetically and non-genetically engineered ESC-derived cardiomyocytes.

Results: The results of this study revealed that both cell lines were suitable for evaluation of DOX cardiotoxicity. This study showed that DOX cardiotoxicity was reduced as detected by beating cardiomyocytes and caspase activity only by pretreatment with dexamethasone (DEX) and enhances cardiomyocyte-specific gene expression.

Conclusion: Therefore, for the general assessment of cytotoxicity, non-genetically engineered ESC-derived cardiomyocytes are sufficient but for the molecular assessment of DOX-induced toxicity, genetically engineered purified ESC-derived cardiomyocytes are necessary.

Keywords: Embryonic Stem Cells, Cardiomyocyte Differentiation, Cytotoxicity, Doxorubicin, Dexamethasone

Ps-24: 1, 25-dihydroxyvitamin D3, As Proper Protector for Endothelial Cells in Multiple Sclerosis Patients

Dehghani L1*, Tahani S, Meamar R

Department of Medical Science, Najafabad Branch, Islamic Azad University, Isfahan, Iran

Corresponding Email: L_dehghani2002@yahoo.com

Objective: Previous publication reported that low-levels of vitamin D are associated with multiple sclerosis (MS). Thereby, strong evidence in MS pathogenesis suggests that endothelial cells (EC) could be harmed in MS. In addition, functional changes in EC and macrovascular injuries lead to blood-brain barrier (BBB) disruption in MS. Current study is the first investigation to elucidate positive influences of vitamin D on ECs surveillance in MS patients.

Materials and Methods: Human umbilical vein endothelial cells (HUVECs) were cultured and then treated with sera from patients who had relapsing remitting MS history and sera from healthy volunteer participants as control group (each group n=15). MTS assay for cell surveillance and Cell Death Detection Kit for evaluating apoptosis were used in this study.

Results: Vitamin D suppressed the induction of apoptosis by the serum of MS patients. This effect was significantly better when it was used before treating HUVECs with the sera of MS. The MTS results showed that pre-administration of 10-7 M 1, 25 (OH)2 D3 significantly increased more ECs survivals. Furthermore, the rate of apoptosis in different groups was assessed by Cell Death Detection Kit that detects inter-nucleosomal degradation of genomic DNA during apoptosis.

Conclusion: Overall it could be concluded that not only vitamin D protects the ECs from apoptosis, also leads into ECs survivals.

Keywords: Endothelial Cell, Multiple Sclerosis, Apoptosis, Vitamin D

Ps-25: Comparative Analysis of Collagen Type I and II Synthesis in Chondrocytes Derived Adipose Derived Stem Cells Versus Natural Articular Chondrocytes

Dehghani L1,2*, Tahani S1, Hashemiben B2, Zamani S3, Esfandiarie E2

1. Department of Anatomical Sciences, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Medical Sciences, Najafabad Branch, Islamic Azad University, Isfahan, Iran

Corresponding Email: L_dehghani2002@yahoo.com

Objective: Due to the limitations of using chondrocytes in cell-based repair of cartilage damages, adipose-derived stem cells (ADSCs) have been considered as an attractive cell source for this purpose. But it has not been clarified if chondrocytes and ADSCs differentiate into
the same cartilage phenotype.

**Materials and Methods:** In this study, ADSCs and human articular chondrocytes were allowed to differentiate in pellet culture for 14 days and were compared their chondrogenic potential. Matrix production was assayed using immunohistochemistry for collagen type I, II.

**Results:** Our results showed significantly increase of collagen type I and significantly decrease of collagen type II in differentiated ADSCs compared to chondrocytes. Histological staining with tuluiden blue showed that both ADSCs and chondrocytes produced glycosaminoglycan.

**Conclusion:** It could be concluded, although less collagen type II and more collagen type I was expressed in ADSCs but, Low production of collagen type II by these cells can also be compensated with optimum conditions like as monolayer culture of these cells.

**Keywords:** Chondrogenic Potential, Chondrocytes, ADSCs, Pellet Culture

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**Ps-26: Optimized Method for Isolation of Stem Cells from Human Dental Pulp**

Ebrahimi B1*, Yaghoobi MM2, Mohammadi Kamal Abadi A3

1. Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Biotechnology, Research institute for Environmental Sciences, International Center for Science, High Technology and Environmental Sciences, Kerman, Iran
3. School of Dentistry, Kerman Medical University, Kerman, Iran

*Corresponding Email: Ebrahimi.b@ssu.ac.ir*

**Objective:** Dental pulp stem cells (DPSCs) are a population of clonogenic and highly proliferative cells derived from enzymatically digested Dental pulp tissue regularly. Providing scaling-up of stem cells at early passages is of importance for regenerative medicine purposes. Typically, two protocols are employed to isolate stem cells from human dental pulp: tissue explants culture and culture of released cells from enzymatically digested pulp tissue. The present study compared two major isolation methods and enhanced efficiency of cell isolation by some modifications.

**Materials and Methods:** Dental pulp was extracted from third molars of 60 healthy subjects. In the first method pulp digested with 1 and 3 mg/mL collagenase/dispase (Roche) for 30 and 60 minutes at 37 °C and released cells obtained using a 70-μm cell strainer for culture, in the second method intact pieces of pulp cultured. In the third method digested pulp pieces from 2 enzyme concentrations for 30 and 60 minutes were immobilized and cultured. The cells and tissues maintained in alpha-MEM supplemented with 20% fetal bovine serum (FBS), 100 U/mL penicillin, 100μg/mL streptomycin, and 25 ng/mL amphotericin B and incubated in humidified incubator with 5% CO2 at 37 °C. The outgrowing and cultured cells monitored visually. In each group cells and colonies were counted and compared.

**Results:** Fifteen different modifications in isolation methods were examined. The results showed that treating pulp segments with 1 mg/mL collagenase/dispase for 30 minutes and culturing immobilized tissues increased the efficiency of cell isolation up to 80% and cells appear in 3-4 days of culture compared with other methods < 20% in 10-15 days.

**Conclusion:** According to the small size of pulp tissue and its low stem cell contents, acquiring substantial quantities of cells in primary culture will facilitate the in vitro expansion and providing adequate production of the stem cells at early passages with minimum risk of losing their “stemness” and aberrant genetic changes for use in research, tissue engineering and regenerative medicine. Optimized method increase efficiency of cell isolation and provides significant quantities of stem cells in primary culture more than other methods.

**Keywords:** Dental Pulp Stem Cells, Cell Culture, Explant Culture

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**Ps-27: Pancreas Extract Induces Differentiation of P19 Embryonic Stem Cells to Insulin-Producing Cells**

Ebrahimi Hafshajani M1-2*, Esmaili F2, Hoshmand F3

1. Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. Research Institute of Biotechnology, Shahrekord University, Shahrekord, Iran
3. Department of Physiology, Faculty of Medical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran

*Corresponding Email: ebrahimimarzieh900@yahoo.com*

**Objective:** So far many research groups are exploring cells to replace destroyed insulin-producing cells in the diabetic patients. One attractive approach is the generation of functional β cells from stem cells. Embryonal carcinoma (EC) stem cells are developmentally pluripotent cells, can differentiate into all cell types under the appropriate conditions. The present study was carried out to examine the effects of mouse pancreas extract (MPE) on production of IPCs from undifferentiated P19 EC cells, using a simple nonselective inductive culture system rather than spontaneous differentiation.

**Materials and Methods:** The cells were grown using low-attachment dishes to induce embryoid body (EB) formation. The resulted EBs cultured in a medium containing 3% fetal bovine serum, supplemented by concentration of 50, 100, 200 and 300 μg/mL MPE.

**Results:** The results showed that P19 cells could differentiate into IPCs and form dithizone-positive cell clusters resemble those of pancreatic islets. P19-derived IPCs were immunoreactive to proinsulin+insulin and insulin receptor beta. Furthermore, expression of the genes related to pancreatic β cell development and function such as, pancreatic and duodenal homeobox 1 (pdx-1), insulin 1 (Ins1) and insulin 2 (Ins2) was observed. When stimulated with glucose, these cells synthesized and secreted insulin in a glucose-regulated manner.
**Conclusion:** The data presented in this study showed that it is possible to generate IPCs from undifferentiated EC cells with the characteristics of pancreatic β cells. The derivation of pancreatic cells from EC cells which are ES cell siblings would provide a valuable experimental tool to study pancreatic development and function.

**Keywords:** Embryonal Carcinoma Cells, Pancreas Extract, Insulin-Producing Cells, Pancreatic β Cell Differentiation, Insulin-Proinsulin

**Ps-28: Changes of Reactive Oxygen Species Levels and DNA Oxidative Damage during The Differentiation of Human Umbilical Cord Mesenchymal Stem Cells into Hepatocyte-Like Cells**

Esmaeli Sh1*, Allameh AA1, Soleimani M2, Emami Aleagha MS1, Rahbarizadeh F1

1. Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

**Corresponding Email:** s.esmaeli.modares.ac.ir

**Objective:** Umbilical cord mesenchymal stem cells (UC-MSCs) are multipotent stem cells that their ability to differentiate into hepatocyte-like cells is demonstrated. The hepatic differentiation is a physiological event that is associated with various molecular and biochemical factors. One of the most important factors affecting on stem cells maintenance and differentiation are reactive oxygen species (ROS) that can be produced during liver regeneration as a result of normal cellular metabolism or generated under pathologic processes. ROS play an important role in carcinogenesis, by generating oxidative DNA damage. Among the markers of DNA oxidative damage, 8-OH dG is the most commonly produced base lesion, and measured as an index of oxidative DNA damage. The current study aims to determine the effect of hepatic differentiation on the levels of ROS production and DNA oxidative damage in hepatocyte-like cells derived from human UC-MSCs.

**Materials and Methods:** Human UC-MSCs were isolated, characterized morphologically, cytofluorometrically, and by their differentiation potential. The hepatic differentiation was performed with DMEM supplemented with 5% FBS, 20 ng/ml HGF, 10 ng/ml FGF-4, 20 ng/ml OSM, and 10-6 M dexamethasone. The expression of markers specific for hepatic was assessed to demonstrate the hepatic differentiation. Then the levels of ROS generation during the differentiation assessed using 2’, 7’-dichloroflurescein diacetate (DCF-DA) assay. To analyze DNA oxidative damage, 8-oxo-dG was measured by 8-OH dG ELISA Kit.

**Results:** Following hepatic differentiation, the MSCs began to show a transition from a fibroblast-like morphology, to a round shape and a cubical morphology. The differentiated cells expressed hepatic-related genes and displayed antibody detectable expression of markers specific for hepatic maturation. UC-MSCs produced ROS at high levels compared with the differentiated cells, but ROS generation was decreased upon the hepatic differentiation. The 8-OH dG levels were not statistically changed during the differentiation; but, its levels were decreased on day 30 of differentiation.

**Conclusion:** Using this protocol of the hepatic differentiation, ROS were generated at low levels, suggesting there was very little DNA oxidative damage during hepatic differentiation.

**Keywords:** Mesenchymal Stem Cells, Hepatic Differentiation, ROS, DNA Oxidative Damage

**Ps-29: Similar Pattern of Epigenetic Bivalent Marks through Reprogramming of Two Human iPSC Lines with Different Origins**

Favaedi R1*, Shahhoseini M1, Molla Mohammadi S2, Baharvand H2

1. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

**Corresponding Email:** m.shahhoseini@royaninstitute.org

**Objective:** Epigenetic affects many biological functions of cells like Pluripotency, differentiation and reprogramming; through molecular mechanisms such as post translational modifications (PTMs) of chromatin. Some PTMs like bivalent marks have important roles in pluripotency maintenance of embryonic stem cells (ESCs) as well as induced pluripotent stem cells (iPSCs). Bivalent marks refer to regions of epigenome with the two concurrent modifications of trimethylated lysine number 4 on histone H3 (H3K4me3) and trimethylated lysine number 27 on histone H3 (H3K27me3). These marks are usually present on regulatory regions of silent developmental genes which should be activated after onset of differentiation.

**Materials and Methods:** Total levels of bivalent marks of H3K4me3 and H3K27me3 were quantitatively compared using chromatin-ELISA technique, in two iPSC lines originated from a differentiated ESC and a fibroblast cell line, respectively.

**Results:** Results clearly showed similarity in pattern of bivalent marks through reprogramming of both differentiated ESC and committed fibroblast cells. While the transcription activating mark of H3K4me3 increased significantly through reprogramming of cells the repressing mark of H3K27me3 decreased during the process.

**Conclusion:** Altogether, it can be concluded that bivalent marks have important role in epigenetic regulation of reprogramming in induced pluripotent stem cells.

**Keywords:** Epigenetic, Pluripotency, Bivalent Marks, iPSCs

**Ps-30: Non-smad TGF-Beta Signaling Pathway Govern Adult Stem Cell State with The Cooperation of SLUG and SOX9**
Fazilaty H1*, Behnam B2

1. Department of Medical Genetics, School of Medicine, Tehran University of Medical Genetics, Tehran, Iran
2. Department of Medical Genetics and Molecular Biology, School of Medicine, Iran University of Medical Genetics, Tehran, Iran

Corresponding Email: h-fazilaty@razi.tums.ac.ir

Objective: Induction of adult stem cell state may open a novel perspective in medicine. Autoregulatory networks involved in determination and maintenance of embryonic stem cell (ESC) state have been investigated. These networks that can be induced by a set of master transcription factors are the source of induced pluripotent stem cells (iPSCs) induction and maintenance. iPSCs are novel powerful tools in regenerative medicine, while their usage overcomes the immunogenicity and ethical problems of ESCs. Although master transcription factors should be present in adult stem cells, our knowledge of such network is poor. Transforming growth factor (TGF)-beta superfamily has shown to be crucial in development, homeostasis and malignancies. In malignancies, TGF-β promotes metastasis by the induction of epithelial to mesenchymal transition (EMT), a process involved in gastrulation, wound healing and induction of cancer stem cells. Interestingly, EMT has been connected to stem cell traits of epithelial tissues by induction of the stemness.

Materials and Methods: By overexpression and knockdown of SLUG and SOX9 TFs, we measure TNC and POSTN expression by real-time PCR and Western blot analysis. SLUG and SOX9 colocalization and co-immunoprecipitation is going to be tested. Stemness analysis in mice is the in vivo confirmation of the regulatory effects of SLUG and SOX9, by fat-pad transplantation of treated cells.

Results: The proposed novel non-smad TGF-beta signaling connects intracellular signaling cascade to the regulation of extracellular matrix proteins involved in stem cell niche, and shows an emerging autoregulatory network in adult stem cells.

Conclusion: Here, a proposed novel non-smad TGF-beta signaling connects intracellular signaling cascade to the regulation of extracellular matrix proteins involved in stem cell niche, and shows an emerging autoregulatory network in adult stem cells. Via this view point, induction of adult stem cells can be a novel area of research with several capacities in regenerative medicine. Regeneration of damaged skin, resected bowel and excised breast can be among achievements of this finding. Key regulatory networks involved that controls stemness in adult stem cells need to be discovered. Master transcription factors that regulate these networks may be the central mystery to be solved.

Keywords: Non-smad TGF-Beta Signaling Pathway, Adult Stem Cells, Master Transcription Factors, Induced Pluripotent Stem Cells, Autoregulatory Network

Ps-31: A Reduction in PGC1α Expression Triggered A Decrease in Fndc5 Expression Level in The Final Stage of Beating Body Formation of Mouse Embryonic Stem Cells

Ghazvini Zadegan F1, 2*, Ghaedi K2, 3, Kalantar SM1, Hashemi MS2, Nasr Esfahani MH2

1. Department of Medical Genetic, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Cellular Biotechnology at Cell Science Research Center, Royan institute for Biotechnology, ACECR, Isfahan, Iran
3. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran

Corresponding Email: f.ghazvini75@gmail.com

Objective: Fibronectin type III domain-containing 5 protein (Fndc5) encodes a membrane protein type 1. Previous studies by our group have shown that Fndc5 expression increased in forming beating bodies from mouse embryonic stem cells (mESCs), representing a novel function for this protein. On the other hand, Bostrom and colleagues identified PGC1α transcriptional co-activator expression induced Fndc5 expression in white adipose tissue (WAT) browning.

Materials and Methods: To understand the relation between PGC1α and Fndc5, in first step expression pattern of PGC1α was investigated under distinct steps of cardiac differentiation by real-timePCR.

Results: Data revealed an elevation in expression level of PGC1α when cardiomyocyte were formed. Thus correlation between PGC1α and Fndc5 was examined by application of a PPARγ antagonist (GW9662) that decreases PGC1α expression. Our results indicated that decreased PGC1α expression significantly reduced expression of Fndc5 in cardiomyocyte.

Conclusion: These data confirm the correlation of Fndc5 and PGC1α in cardiogenesis which needs further verification.

Keywords: Fibronectin Type III Domain-Containing 5 Protein (Fndc5), PGC1α Transcriptional Co-Activator, Cardiogenesis

Ps-32: Survey Specifics of Nucleus Pulposus Cells of Human Intervertebral Disc in Chitosan-Gelatin and Alginate Scaffolds

Ghorbani M1*, Bahramian H2, Hasheimbenni B3, Karimir Z2

1. School of New Modern Technology, Tehran University of Medical Sciences, Tehran, Iran
2. School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Corresponding Email: dr.ghorbani62@yahoo.com

Objective: Low back pain is a major social problem nowadays. Intervertebral disc herniation and central degeneration of disc are two major reasons of low back pain that occur because of structural impairment of discs. Intervertebral disc includes the annulus fibrosus, transitional region, and nucleus pulposus (NP). NP forms the central nucleus of the disc. Reduction of cell count and extracellular matrix, especially in NP, causes disc degeneration. Different scaffolds (natural and synthetic)
have been used for tissue repairing and regeneration of intervertebral disc in tissue engineering. Most scaffolds have biodegradable and biocompatible characteristics and also prepare a fine condition for proliferation and migration of cells. Because degeneration is result of decrease ECM and aggrecan is important proteoglycan in NP tissue of IVD thus goal of this study was survey and comparison rate of secreted aggrecan, morphology of NP cells and proliferation and viability of NP cells in alginate and chitosan-gelatin scaffolds.

**Materials and Methods:** NP cells were derived by enzymatic hydrolysis of collagenase from NP tissue of patients undergoing open surgery for discectomy in Alzahra Hospital (Isfahan, Iran). Chitosan was blended with gelatin and glutaraldehyde was used for cross linking of the two polymers. Then, alginate scaffold was prepared. After approving the NP cells by flow cytometry of cytokeratin 18 marker, a cellular suspension with 4 × 10⁵ cells was transferred to each scaffold and cultured for 21 days. Cell viability and proliferation were investigated by trypan blue and methyl thiazolyl tetrazolium (MTT) assay. A scanning electron microscope (SEM) was used to assert the porosity and to survey the structures of the scaffolds. ELISA assay was used for determination of aggrecan secretion by NP cells seeded in chitosan-gelatin and alginate scaffolds.

**Results:** We can use flow cytometry of cytokeratin 18 markers for recognition of NP cells. 80% of NP cells express CK18. MTT assay demonstrated that cell viability on the third day had significant difference with the first day in both scaffolds. There was also a significant reduction in cellular viability from day 3 to day 21. ELISA assay showed had significant difference with the first day in both scaffolds. There was also a significant increase in produced of aggrecan from day 3 to day 21 (p<0.001). Alginate scaffold had higher secretion of aggrecan from day 3 of day 21. Results of cell count showed that mean difference between cell counts in alginate scaffold was significantly more than chitosan-gelatin scaffold (p<0.001).

**Conclusion:** Flow cytometry of cytokeratin 18 can be used as a method for recognition of NP cells. Compared to chitosan-gelatin scaffold, alginate scaffold prepared a better condition for proliferation of NP cells, maintain of morphology of cells and produce of aggrecan. The results of this study suggested that alginate scaffold could be useful in in-vivo studies and treatment.

**Keywords:** Intervertebral Disc, Tissue Engineering, Degeneration, Scaffold, Chitosan, Gelatin, Algin, Cytokeratin 18, Aggrecan

**Ps-33: Effect of Pentoxifylline in The Presence of Stauorosporine on Neuronal Differentiation in Mouse Bone Marrow Mesenchymal Stem Cells**

**Goodarzdashti A*, Azadbakht M, Zahleh H, Pourmoradi M**

Department of Biology, Faculty of Basic Sciences, Razi University, Kermanshah, Iran

**Corresponding Email:** az.dashty@gmail.com

**Objective:** The in vitro generation of neural-like cells from stem cells is a promising approach to produce cells suitable for neural tissue repair and cell-based replacement therapies in neurological damages. Available methods to promote stem cells differentiation towards neural lineages attempt using the various compounds had been partly successful. This study was undertaken to evaluate the effects of different concentrations of pentoxifylline in the presence of stauorosporine on neuronal differentiation in mouse bone marrow mesenchymal stem cells.

**Materials and Methods:** In the present study, mouse mesenchymal stem cells are isolated from an aspirate of bone marrow harvested from the tibia and femoral marrow compartment. Cells were cultured in DMEM culture medium supplemented with 10% FBS for 72 hours. After the first passage, in experiment group cells were pre-pretreated with different concentrations of pentoxifylline (10⁻⁶, 10⁻⁵, 10⁻⁴M, treatments I, II, III; respectively), Then cells were treated in the same concentrations of pentoxifylline in the presence of 100nM stauorosporine. Non-pretreated cells with pentoxifylline were considered as control group. Neurigial differentiation was performed by total neurite length measurement GFAP and β-tubuline immunocytochemistry staining.

**Results:** After 12 hours Total neurite length measurements after 6 hr in control group, treatments I, II and III was 286.839, 309.166, 330.49, 334.509 μm; respectively. Pentoxifylline at either of low or high concentration resulted in increase of neurite elongation (p<005). Pentoxifylline at either of low or high concentration enhanced Tubulin Beta III and GFAP protein expression compared with control (p<005). Immunocytochemistry data showed that pentoxifylline in treatment II increased Tubulin Beta III and GFAP protein expression compared with other treatments in experiment group.

**Conclusion:** It suggests that pentoxifylline stimulate the neuronal differentiation by enhancing neurite elongation and neuronal protein expression in mouse bone marrow mesenchymal stem cells.

**Keywords:** Pentoxifylline, Neuronal Differentiation, Neurite Outgrowth, mBMSCs

**Ps-34: Effect of Pentoxifylline in The Presence of Stauorosporine on Mouse Bone Marrow Mesenchymal Stem Cells**

**Goodarzdashti A*, Azadbakht M, Pourmoradi M, Zahleh H**

Department of Biology, Faculty of Basic Sciences, Razi University, Kermanshah, Iran

**Corresponding Email:** az.dashty@gmail.com

**Objective:** Bone marrow mesenchymal stem cells can be differentiated into neuronal and glial cell types under appropriate experimental conditions. Pentoxifylline induce its effects by several mechanisms including translocation...
of extracellular calcium, increasing in cAMP caused by inhibition of phosphodiesterases. This study was undertaken to evaluate the effects of different concentrations of pentoxifylline in the presence of staurosporine on mouse bone marrow mesenchymal stem cells (mBMSCs).

**Materials and Methods:** In the present study, mouse mesenchymal stem cells are isolated from an aspirate of bone marrow harvested from the tibia and femoral marrow compartment. Cells were cultured in DMEM culture medium supplemented with 10% FBS for 72 hours. After the first passage, in experiment group cells were pretreated with different concentrations of pentoxifylline (10-6, 10-5, 10-4M, treatments I, II, III; respectively). Then cells were treated in the same concentrations of pentoxifylline in the presence of 100 nM staurosporine. Non-pretreated cells with pentoxifylline were considered as control group. The viability of cells was assessed by neutral red uptake assay and cell death was performed by PI/Hoechst staining assay.

**Results:** The viability of cells in control group was higher than experiment group in all treatments and the lowest viability was in experiment group, treatment III (81.75%), (p<0.05). The highest cell death after 6, 12 and 24 hr observed in treatment III (28.61%, 33.17% and 41.98%; respectively) and the lowest cell death observed in control group 917.12%, 17.35% and 18.9%; respectively) (p<0.05).

**Conclusion:** According to our results, pentoxifylline increased cell death and in mBMSCs in a time and dose dependent manner.

**Keywords:** Pentoxifylline, Staurosporine, Cell Death, mBMSCs


Goudarzi N

Department of Anatomy, Lorestan University of Medical Science, Khoramabad, Iran

*Corresponding Email: ngoodarzy@yahoo.com*

**Objective:** Many researchers have focused on transplantation of pluripotent stem cells for repair of peripheral nerve injury. The umbilical cord matrix is rich source of mesenchymal stem cells that doesn’t stimulate immune system. The cells can be proper for treatment of nerve tissue injury. We examine the effects of HUMSC transplantation after transaction of sciatic nerve in rat by histological study.

**Materials and Methods:** In this research, 30 adult male wistar rats (200-250 g) divide in to three groups randomly (n=10 in each group). A(control group) silicon tube, B(fibrin gel) silicon tube filled with fibrin gel without the cells, C(experimental group) silicon tube filled with fibrin gel seeded with HUMSC. In every group left sciatic nerve cut 10 mm and use 12 mm silicon tube as guide the nerve. In experimental group, HUMSC cultured and transplanted into tube. Eight weeks after the surgical procedure was assessed regeneration of sciatic nerve by histological study.

**Results:** Histological assessment showed a significant difference between the experimental groups compared with other groups. Histological measures were significantly better in the HUMSC transplantation group compared with other groups (p<0.05).

**Conclusion:** Histological evaluation confirms that transplantation of HUMSCs improves repair of peripheral nerve injury.

**Keywords:** Peripheral Nerve Repair, Stem Cell

**Ps-36: Higher Expression of Signal Transducers and Activators of Transcription-3 (STAT-3) in Gastric Cancer Stem Like Cells (GCSCs)**

Hajimoradi M*, Hassan ZM1, Ebrahimi M2

1. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Stem Cells and developmental Biology at Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

*Corresponding Email: hajimoradi.m@live.com*

**Objective:** STAT-3 transcription factor participates in some biological phenomenon including inflammation, anti-tumor immunosuppression, cellular proliferation and differentiation. Moreover, it helps to maintain pluripotency of embryonic stem cells and there are some recent evidences of its footprint in stemness properties of cancer initiating cells. In the present study, the expression of STAT-3 was evaluated in the spheroid bodies derived from MKN-45 cell line as cancer stem like cells and the results compared to parental cells.

**Materials and Methods:** Spheroids obtained from non adhesive culture of cells in serum free media supplemented with EGF, bFGF (20 ng/ml) and B27 (2%). Gastric-spheres were characterized by expression of CD24, CD44, CD133, EpCam, CD71, CD73, CD90 as putative CSCs markers; Nanog, Oct-4, Sox-2, Klf-4 and c-Myc as stemness related genes and drug resistancy to DTX. Finally, the expression of STAT-3 tested by immunofluorescence staining and real-time PCR. Parental cells were used as control group.

**Results:** Gastric-spheres (passage 4) showed higher expression of CD44, CD24, CD71, and over expressed Nanog, Oct-4, Sox-2, Klf-4 and c-Myc genes than parental cells. Immunostaining of STAT-3 demonstrated that both spheroid cells and parental cells expressed STAT3 at protein level, however, its mRNA level in gastro-spheres was higher than parental cells.

**Conclusion:** Spheroid formation provide an applicable method to isolate cancer stem like cells from MKN-45 cell line and the findings if support by further examinations may imply to roles of STAT-3 in GCSCs features, as recently seen in brain and glioblastoma CSCs.

**Keywords:** Gastric Cancer, Cancer Stem Cells, STAT-3
Ps-37: Evaluation of Toll Like Receptors Expression in Cardiomyocyte Differentiation from Human Embryonic Stem Cells

Hashemzadeh MR1-2*, Aghdami N1-3, Aghajanpour S4, Mazidi Z1, Fonoudi H2, Aflatoonian R3

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Cellular and Molecular Research, Royesh Stem Cell Biotechnology Research Center, Mashhad, Iran
3. Department of Regenerative Medicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Corresponding Email: hashemzadeh.m.reza@gmail.com

Objective: The transmembrane receptor family of Toll-like receptors (TLRs) is expressed in immune cells and cardiac muscle. To date, several TLRs (numbered 1-11) have been identified in humans. TLRs may play a role in initiating early inflammatory and functional responses to danger signals arising from ischemia-reperfusion and inflammatory stimuli. Human embryonic stem cells (hESCs) have the potential to provide an unlimited source of cardiomyocytes, which are invaluable resources for drug or toxicology screening, medical research, and cell therapy.

Materials and Methods: Royan H5 and Royan H6 hESCs lines were used in this study. Suspension culture of hESCs was performed according to a recently published protocol. Differentiation of the cells into cardiomyocytes in suspension was performed according to the Laflamme et al. protocol with some modifications. In this study, expression of TLR2, TLR3, TLR4, TLR5 and TLR9 was evaluated by RT-PCR and Q-PCR during cardiomyocyte differentiation on day 8, 14 and 25 (mature cardiomyocyte).

Results: According to Q-PCR data, TLR9 expression has increased and TLR5 expression has decreased in linear pattern during cardiomyocyte differentiation. Expression pattern of TLR2 and TLR4 was same in sigmoid shape. In both of them, expression level was lowest in mature cardiomyocyte. Also, about TLR3 expression, our data was shown sigmoid shape but the lowest expression related to 8th day after differentiation.

Conclusion: The goal of this study was to investigate the ability TLR expression in cardiomyocyte differentiation from human embryonic stem cells. According to results, all of TLRs in this study have been expressed in cardiomyocyte in variable level. TLRs expression related to inflammatory responses, ischemia and contractility. On the other hand, myocardial infarction and all of myocardial ischemia diseases have inflammatory reasons. Hence, evaluation of TLRs expression during cardiomyocyte differentiation is indispensable in cardiac cell based therapy.

Keywords: Human Embryonic Stem Cells, TLRs, Cardiomyocyte, Differentiation

Ps-38: Effective Megakaryocytic Differentiation of Umbilical Cord Blood Hematopoietic Stem/Progenitor Cells Using A Two-Phase Protocol

Hatami J1, Andrade PZ, Cabral JMS, da Silva CL, Ferreira FC

Department of Bioengineering, Institute for Biotechnology and Bioengineering(IBB), Instituto Superior Tecnico (IST), Lisbon, Portugal
Corresponding Email: javad.hatami@ist.utl.pt

Objective: There is a high need for the ex-vivo expansion of Hematopoietic stem and progenitor cells (HSC/HPC), especially those from the umbilical cord blood (UCB) which are limited in number, for their extensive use in hematopoietic transplantation. Likewise, considering that platelets do not withstand refrigeration and having short lifetime of 5 days, a high demand for cells with megakaryocytic potential exists aiming at the clinical scale production of platelets for transfusion. However the complexity of hematopoiesis, the heterogeneity of culture population and the different combination of culture parameters has impaired to establish an efficient protocol to be used for platelet production from UCB. Thus, we aimed to develop a two-phase ex-vivo protocol for expansion and differentiation of HSC/HPC from UCB towards megakaryocyte lineage using a static co-culture system.

Materials and Methods: HSC/HPC from UCB were co-cultured with mesenchymal stem cells from bone marrow (BM-MSC) in a serum free medium with a cytokine cocktail including TPO, SCF, FLT3, b-FGF (50, 60, 55 and 5 ng/mL, respectively). After 7 days of expansion, the hematopoietic cells were retrieved and submitted to a differentiation protocol using IMDM medium supplemented with 10% fetal bovine serum and a cocktail of TPO and IL3 (100 and 10 ng/mL, respectively).

Results: HSC/HPC were co-cultured with BM-MSC for 7 days, reaching a 20 ± 5 fold increase in CD34+ cell number. By the end of differentiation phase, more than 35 % of cells population expressed megakaryocyte lineages surface maker (CD41+) and we were able to produce 50 Megakaryocytes per input CD34+ cells. Inverted and electron microscopy images confirmed megakaryocyte and platelet morphology.

Conclusion: HSC/HPC from UCB were successfully expanded in a static co-culture system, phenotype and morphological analysis suggested effective differentiation of HSC/HPC towards the megakaryocyte lineage.

Keywords: UCB, Megakaryocyte, Differentiation, Platelet


Hekmatimoghaddam Sh1-3, Pourrajab F1-3*, Forouzanna SK1, Ebrahimii H1, Tajik Kord M3
1. Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Regenerative Medicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Corresponding Email: javad.hatami@ist.utl.pt
2. School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Clinical Biochemistry and Molecular Biology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Corresponding Email: negin_h7@yahoo.com

Objective: MicroRNAs (miRs) have the potential to control stem cells fate decisions through regulation of gene expression. miR-210 is one of this agents that is shown to be associated with VEGF during angiogenesis in endothelial cells and promote survival and number of vascular colony forming units of haematopoietic stem cells (HSCs). Accordingly, we hypothesized that over expression of miR-210 may also promote human Wharton’s jelly stem cells (hWJSCs) proliferation, while inhibit the cells senescence and apoptosis through targeting the genes involved in these processes.

Materials and Methods: We evaluate the direct effect of over-expression of miR-210 in expansion potential and on the proliferation rate of hWJSCs. On day 5 of expansion, cells were seeded in media and transfected with miRs at a final concentration of 160 nM using Lipofectamine. To silence miR-210 expression, cells were treated with specific Anti-miR Inhibitor, hsamiR- 210.

Results: After 48 hours incubation, hWJSCs exhibited effectively expanded potential (15- to 20-fold) in culture medium containing miR-210, while miR-210 inhibitor abrogated significantly the proliferator capacity of treated cells.

Conclusion: Collectively, these observations highlight a critical role for microRNAs in enhancing the proliferation property of expanded cells, and identify miR-210 as a potential molecule and a therapeutic target to enhance the proliferation rate of hWJSCs. On day 5 of expansion, cells were seeded in media and transfected with miRs at a final concentration of 160 nM using Lipofectamine.

Keywords: MicroRNA, miR-210, Human Wharton’s Jelly Stem Cells, Proliferation

Ps-40: Study of The Relation between rs11677451 and rs 2030259 Polymorphic Markers in Jhdm2a Gene and Male Infertility

Hoghoughi N*, Hojati Z

Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Iran
Corresponding Email: negin_h7@yahoo.com

Objective: JHDM2A is a histone demethylase that specifically demethylates mono- and di-methylated histone H3 lysine 9. JHDM2A (JmjC-domain-containing histone demethylase 2A, also known as JMJD1A and KDM3A) is essential for spermatogenesis. JHDM2A also binds to and controls the expression of transition nuclear protein 1 (Tnp1) and protamine 1 (Prm1) genes, the products of which are required for packaging and condensation of sperm chromatin.

Materials and Methods: Two Non-synonymous SNPs, rs11677451 and rs2030259 have been selected by Bioinformatics studies. Four primers were designed by using Oligo primer software. Genomic DNA was extracted from the blood of 50 control samples and 100 patients with azoospermia and oligozoospermia, then jhdm2a gene was amplified. Finally the samples analyzed by agarose gel.

Results: So far we have observed bands in considered length due to designed primers, for patient and control samples, and its relationship with male infertility is under investigation.

Conclusion: This study has focused on polymorphic markers of jhdm2a gene to evaluate its association with male infertility. The association reported in this study will be necessary to confidently validate these SNPs and identify novel SNPs associated with male infertility that can have therapeutic purposes.

Keywords: Histone Demethylase, JmjC-domain-containing Histone Demethylase, Nuclear Protein, Protamine

Ps-41: The Effect of Low Intensity Ultrasound on Mesenchymal Stem Cells

Hormozi Moghaddam Z

Department of Medical Physics, Modares University, Tehran, Iran
Corresponding Email: hormozizeinab20@yahoo.com

Objective: Ultrasound have a wide application in medical. Because ultrasound can be characterized in many therapies, diagnostics, research and laboratory use. In this study, the effects of ultrasound on human mesenchymal stem cells has been studied. This study classified the Republic of ultrasound on the growth, proliferation or differentiation of cells in recent studies.

Materials and Methods: This review is from studies on the effects of ultrasound on mesenchymal stem cells in recent years.

Results: An example of the results of the studies presented in the conditions is as follows: the effect of LIUS on Factor β-Mediated Chondrocyte Differentiation when low-intensity US was applied for 20 minutes every day to the TGF-β-treated cell pellets, chondrocyte differentiation was enhanced, and the effects of LIUS on chondrogenic differentiation of bone marrow-derived mesenchymal stem cells (BM-MSC), total collagen and glycosaminoglycan (GAG) increased more significantly in the US-stimulated group than in the control. The mechanical effect generated by US for the stimulation of mesenchymal stem cells (MSCs). MSCs were cultured on flexible cell culture membranes and stimulated by US for 10 minutes daily with acoustic intensities of 0, 6, 13.5, and 22.5 W/cm². The results validate that US is able to influence the cell matrix interaction.

Conclusion: Ultrasound can be used as an alternative for the characterization of mesenchymal stem cells can be introduced. The need for the study of stem cells is also important.

Keywords: Low-intensity Ultrasound, Chondrogenesis, Mesenchymal Stem Cell
Ps-42: The Effect of Retinoic Acid in Differentiation of Male Mouse Adipose Tissue Derived-Mesenchymal Stem Cells into Germ Cells

Hoseinzadeh Shirzeily M*, Sobhani A, Pasbakhsh P, Amidi F

Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Corresponding Email: Sobhania@ums.ac.ir

Objective: Recent publications regarding to differentiation of stem cells to germ cells have motivated researchers to make new approaches to infertility. In vitro production of germ cells not only provides new approaches to infertility, but also improves understanding differentiation process of male and female germ cells. Because using embryonic stem cells for this purpose has been associated with tumorogenesis and also ethical criticisms, the mentioned cells were suggested to be replaced with some adult multipotent stem cells. To find appropriate non invasive source replacement for embryonic stem cells in this study we designed to evaluate differential potentials of adipocyte derived stem cells (ADMSCs) to germ like cells.

Materials and Methods: To find differentiation capability, after providing purified ADMSCs differentiation to osteoblast and adipocyte was confirmed by using appropriate culture medium. Superficial markers for mesenchymal stem cells (expression of CD90 and CD73 and non-expression of CD45 and VEGFR2) were investigated by flowcytometry to confirm mesenchymal lineage production. The cells were differentiated to germ cells in mediums containing Retinoic Acid for 7 days. To evaluate germ cells characteristic markers (Mvh, Dazl, Stra8, Scp3) flow-cytometry, immuno florescence and RT-PCR were used.

Results: Presentation of stem cell superficial markers (CD90, CD73) and absence of endothelial and blood cell markers (VEGFR2, CD45) were confirmative for mesenchymal origin of these cells. The cells were able to differentiate into osteoblast and adipocyte cells. This fact was representative for multipotential entity of the examined cells. The flow cytometry, immuno florescence and RT-PCR results showed remarkable expression of germ cells characteristic markers (Mvh, Dazl, Stra8, Scp3).

Conclusion: By this study, it was found that germ cell markers were expressed in ADMSCs after adding exogenous Retinoic Acid into culture medium.

Keywords: Mesenchymal Stem Cells, Infertility, Germ Cells, Retinoic Acid

Ps-43: Evaluation of Viability and Proliferation of Wharton’s Jelly Mesenchymal Stem Cell in Different Concentrations of Alginate Scaffold

Hosseini SM1, 2, 3, 4*, Azari H2, Samimi N1, 2, 3

1. Students’ Research Committee, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Cell and Molecular Research Club, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Anatomical Sciences, Laboratory for Stem Cell Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Anatomical Sciences, Laboratory for Stem Cell Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding Email: hoseini2010m@gmail.com

Objective: The neurosphere assay is usually used to culture neural stem cells (NSCs). In this system, cells are under the least physical interaction with their environment. Using a more solid matrix might affect cellular behavior in terms of proliferation and differentiation that could benefit selection of cells with different properties. Alginat is a polysaccharide that is used in 3D cultures.
Here, we evaluated NSCs expansion in different alginate concentrations (0.25, 0.5, and 1 %).

**Materials and Methods:** First, NSCs from the E-14 mouse brain were cultured in different Alginate concentrations (0.25, 0.5 and 1%) and a control group. After 5-6 days, the cultures were evaluated for neurosphere growth and the sphere forming frequency was determined for each condition.

**Results:** Evaluating the cultures from different groups showed that neurospheres could grow in all conditions but the sphere forming frequency was the highest in control comparing to the Alginate groups. In Alginate groups, it was revealed the higher the Alginate concentration, the lower the sphere forming frequency. Furthermore, all spheres from both control and Alginate groups could be serially passed. Comparing the size of spheres, it was evident that the spheres in higher concentration of Alginate were larger than the control.

**Conclusion:** Our study shows that the mechanical effects of alginate 3D scaffold reduce the sphere forming frequency of neural stem cells. At the same time growing NSCs in Alginate scaffold results in larger spheres that could be due to selection of bona fide NSCs in Alginate culture comparing to the control.

**Keywords:** Neural Stem Cell, Alginate Scaffold, Sphere Forming

**Ps-45: Assessment of The Possibility of ERK1/2 Effect on Fndc5 Expression during Neural Differentiation of Mouse Embryonic Stem Cells**

Hosseini Farahabadi SS*, Karbalaie Kh2, Baharvand H3, 4, Ghaedi K1, 2, Nasr Esfahani MH2

1. Cell and Molecular Biology Division, Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
2. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

**Corresponding Email:** samaneh.hosseini.20@gmail.com

**Objective:** Fibronectin type III domain containing 5 protein (Fndc5) is a protein with unspecified role in neural differentiation. Early studies have indicated during neural differentiation of mouse embryonic stem cells (mESCs), Fndc5 expression enhanced specifically in neural precursors while Fndc5 knockdown, declined the neural differentiation efficiency suggesting a presumable role for Fndc5 in neural differentiation. To look further on the signaling pathway responsible for controlling Fndc5 expression during neural differentiation, MAPKs signaling pathways, which are a highway controlling most processes of differentiation were chosen to be studied.

**Materials and Methods:** Fndc5 expression assessment was carried out by means of Real-time PCR and MAPKs activity was assessed by means of quantitative western blot.

**Results:** The ERK1/2 MAPKs activity indicated a positive trend line correlation between the ERK1/2 activation and Fndc5 expression. Blockage of the ERK1/2 activation with respective specific inhibitor PD0325901 during neural differentiation, carried out to help us to assess whether it has an effect on Fndc5 expression.

**Conclusion:** In case of a relationship, more strict and detailed studies should be carried out based on this result to find out the signaling pathway controlling Fndc5 expression because it may have importance in treatment of neurodegenerative disorders.

**Keywords:** Fndc5, Signaling Pathway, ERK1/2, Neural Differentiation, Mouse Embryonic Stem Cells

Ilic D*, Stephenson E1, Wood V1, Jacquet L1, Stevenson D1, Petrova A2, Kadeva N1, Codognotto S1, Patel H1*, Semple M1

1. Embryonic Stem Cell Laboratories, Guy’s Assisted Conception Unit, Division of Women’s Health, King’s College School of Medicine, London, UK
2. St John’s Institute of Dermatology, Dermatology Research Laboratories, Guy’s Hospital, King’s College School of Medicine, London, UK
3. Assisted Conception Unit, Guy’s and St Thomas’ Hospital Trust, London, UK

Corresponding Email: dusko.ilic@kcl.ac.uk

Objective: Human embryonic stem (hES) cells hold great potential for cell therapy and regenerative medicine because of their pluripotency and capacity for self-renewal. The conditions used to derive and culture hES cells vary between and within laboratories depending on the desired use of the cells. Until recently, stem cell culture has been carried out using feeder cells, and culture media, that contain animal products. Recent advances in technology have opened up the possibility of both xeno-free and feeder-free culture of stem cells, essential conditions for the use of stem cells for clinical purposes.

Materials and Methods: The derivation of hES cells at King’s College London (London, UK) is under license from the UK Human Fertilisation and Embryology Authority (HFEA; research license number R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference 06/Q0702/90). No financial inducements are offered for donation. In accordance with HFEA regulations, a sample of each line that is derived is deposited in the UK Stem Cell Bank for distribution to academic and research centers internationally. Fresh embryos for the derivation were obtained from the Guy’s Assisted Conception Unit (ACU; London, UK) pre-implantation genetic diagnostics (PGD) program. Cryopreserved embryos no longer wanted for therapeutic use by patients were from both Guy’s ACU as well as external units, obtained through the human embryonic stem cell co-ordinator’s (hESCCO) network.

Results: Detailed protocols are developed for deriving human embryonic stem cell lines in xeno-free conditions from cryopreserved embryos. From derivation to cryopreservation of fully characterized initial stocks takes 3-4 months. These protocols served as the basis for standard operating procedures (SOPs), with both operational and technical components, that we set to meet good manufacturing practice (GMP) and UK regulatory body requirements for derivation of clinical-grade cells.

Conclusion: As such, these SOPs were used in our current GMP-compliant facility to derive hESEs cell lines ab initio, in an animal product-free environment; these lines are suitable for research and potentially for clinical use in cell therapy. So far, we have derived eight clinical-grade lines, which will be freely available to the scientific community after submission/accession to the UK Stem Cell Bank.

Keywords: Human Embryonic Stem Cells, Clinical Grade, Cell Therapy

Ps-48: Oxytocin Improves Neuronal Differentiation of Adipose Tissue-Derived Stem Cells

Jafarzadeh N*, Taha MF, Javeri A, Khaleghi M

Department of Nano BioMaterials and Tissue Engineering, National Institute of Genetics Engineering and Biotechnology, Tehran, Iran

Corresponding Email: mftaha@nigeb.ac.ir

Objective: The aim of the present study was to investigate the effects of oxytocin on neural differentiation of mouse adipose tissue-derived stem cells (ADSCs).

Materials and Methods: ADSCs were isolated from inguinal adipose tissue of 8 to 10-week old mice. Third-passaged ADSCs were cultured in DMEM containing 15% Knockout Serum Replacement (KoSR) to induce neural differentiation. 10-6, 10-7 or 10-8 M oxytocin was added to culture medium on the first or 8th day of differentiation. After two weeks of differentiation, the expression of neural-specific markers was evaluated by RT-PCR and immunocytochemistry. Moreover, the expression levels of Nestin, NSE, NeuN, NEFL and Synaptophysin mRNAs were compared between groups by quantitative real-time PCR.

Results: ADSCs expressed oxytocin and oxytocin receptor mRNAs before and after differentiation. Two weeks after differentiation, control and oxytocin-treated ADSCs expressed PAX6, Nestin, NSE, NeuN, NEFL and PCNA mRNAs. Tyrosine hydroxylase (TH) and GAD2 were also expressed in the differentiated ADSCs in all groups. ChAT was expressed at the presence of 10-7 M oxytocin. The expression of GFAP in the differentiated ADSCs was negative. As detected by quantitative real-time PCR, the optimal dose and the best time point for oxytocin treatment were 10-8 M and the eighth day of differentiation, respectively. In this condition, differentiated cells showed the highest expression levels of NSE and NEFL. The presence of TuJ1/β-tubulin III, MAP2 and NEFL proteins in the ADSC-derived neurons was confirmed by immunocytochemistry.

Conclusion: These results demonstrated that oxytocin treatment can promote neural differentiation of the ADSCs, in a dose-dependent and time-dependent manner. Oxytocin has a role in neural development, and this may have significant implications in neural regeneration after injury.

Keywords: Oxytocin, Adipose Tissue-derived Stem Cells, Differentiation, Neuron

Ps-49: Effect of Hydrostatic Pressure in The Presence of Staurosporine on Mouse Bone Marrow Mesenchymal Stem Cells

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Javanmard F*, Azadbakht M, Zhaleh H

Department of Biology, Faculty of Basic Sciences, Razi University, Kermanshah, Iran
Corresponding Email: azadbakhtm_tmu@yahoo.com

Objective: Bone marrow mesenchymal stem cells (BMSCS) are multipotent, and proliferate freely in vitro to undergo self-renewal and differentiation into multiple non-hematopoietic cell lineages such as neuronal cells. The cells in the body are continuously exposed to a complex mechanical environment. Hydrostatic pressure as a mechanical force is effective in regulating the neural differentiation in many type of cell lines. The aim of the present study was to evaluate the effect of hydrostatic pressure in the presence of staurosporine on mouse bone marrow mesenchymal stem cells.

Materials and Methods: Mouse mesenchymal stem cells are isolated from an aspirate of bone marrow harvested from the tibia and femoral marrow compartment. Cells cultured in DMEM culture medium supplemented with 10% FBS for 72 hours. After the first passage cells were cultured in treatment medium containing 100 nM of staurosporine for 4 h, than the cells were affected by hydrostatic pressure (0, 25, 50, 100 mm Hg). The cell viability and cell death were assessed using trypan blue and Hoechst/PI staining.

Results: Our results indicate that the highest percentage of cell viability was 84% in the 0 mm Hg hydrostatic pressure. The lowest percentage of cell viability was 52% in the 100 mm Hg hydrostatic pressure. The highest percentage of cell death was 52% in the 100 mm Hg hydrostatic pressure after 24 hours and the lowest percentage of cell death was 17% in the 0 mm Hg hydrostatic pressure.

Conclusion: Based on these observations, we conclude that the viability of cells in treatments reduced according to increasing in hydrostatic pressure and with passing time. Our data revealed that the cell death in treatments increased according to increasing in hydrostatic pressure and with passing time.

Keywords: Hydrostatic Pressure, Bone Marrow Mesenchymal Stem Cells, Mouse

Ps-50: Effect of Hydrostatic Pressure in The Presence of Staurosporine on Neurite Outgrowth in Mouse Bone Marrow Mesenchymal Stem Cells

Javanmard F*, Azadbakht M, Zhaleh H

Department of Biology, Faculty of Basic Sciences, Razi University, Kermanshah, Iran
Corresponding Email: javanmard.takvin@yahoo.com

Objective: Bone marrow mesenchymal stem cells (BMSCS) are pluripotent cells that can differentiate into many types of cell lines including; neural cells. Mechanical forces are an important factor on nerve regeneration. It also should be noted that the mechanical stimulation could induce not only proliferation but also differentiation of MSCs. The cells in the body are continuously exposed to a complex mechanical environment. Hydrostatic pressure as a mechanical force, play a key role in regulating the neural differentiation in many type of cell lines. In this study we investigated the effect of hydrostatic pressure on neurite outgrowth and expression of neural markers in staurosporine-induced neural differentiation in mouse bone marrow mesenchymal stem cells.

Materials and Methods: BMSCs were isolated from rat bone marrow based on their capacity to adhere to plastic culture surfaces and cultured in DMEM culture medium supplemented with 10% FBS. After the first passage cells were cultured in treatment medium containing 100 nM of staurosporine for 4 hours, than the cells were affected by hydrostatic pressure (0, 25, 50, 100 mmHg). The neurite outgrowth and expression of B-tubulin III and GFAP proteins was analysed by immunocytochemistry.

Results: Our results showed that total neurite length were 178.89, 222.98, 170.17, 120.19 µm in 0, 25, 50, 100 mm Hg hydrostatic pressure treated cells; respectively. Total neurite length increase in 25 mm Hg hydrostatic pressure treated cells and decreased in 100 mm Hg hydrostatic pressure treated cells (p<0.05). Immunocytochemistry analysis revealed that expression of B-tubulin III and GFAP proteins was strongly increased in 25 mm Hg hydrostatic pressure treated cells.

Conclusion: According to our results, it is seem that hydrostatic pressure can improve length of neurite in mouse bone marrow mesenchymal stem cells in an amount dependent manner. Therefore hydrostatic pressures a mechanical force can be used for improvement of neural differentiation in bone marrow mesenchymal stem cells.

Keywords: Neural Differentiation, Immunocytochemistry, Hydrostatic Pressure, Bone Marrow Mesenchymal Stem Cells, Mouse

Ps-51: The Effects of Extremely Low-Frequency Pulsed Electromagnetic Fields on Osteogenic Differentiation of Mesenchymal Stem Cells

Jazayeri M, Shokrgozar MA, Haghighipour N*

National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
Corresponding Email: haghighipour@pasteur.ac.ir

Objective: Osseous tissue Repair and regeneration is a big challenge in orthopedic and maxillofacial surgery. Surgical interferences are usually needed in most abnormalities like trauma and infections and Bone grafts are usually used as a treatment for such diseases. Tissue engineering makes it possible to cure different tissue abnormalities with no need to surgery by using the autologous grafts which are the stem cells of the patient that undergone some types of stimulation. Recently, Applying electromagnetic fields enter the usage of physical signals in the field of tissue engineering. Transmission of physical signals is related to change in structural organelles and proteins will translate to biochemical signals. Extremely low magnetic field (ELF) with low frequencies
can mediate biologic responses. Electromagnetic waves can increase intra cellular calcium and cause activation of calmodulin and increase in expression of osteogenic marker genes. So magnetic fields could play decisive role in promotion of differentiation pathways.

**Materials and Methods:** Four weeks old mature rats were anesthetized and bone marrow stem cells were isolated and cultured in α-MEM medium containing 15% FBS. Flow-cytometry (CD45 and CD90) and multipotent tests confirmed the identity of the isolated cells. Pulsed electromagnetic field (PEMF) was applied with 0.2mT intensity and 15Hz frequency; 6 hours per day. The test groups were included of chemical, magnetic and magnetic/chemical. In magnetic/chemical group, osteogenic induction medium (containing 1 μM dexamethasone, 10 mM β-glycerophosphate and 0.1 mM ascorbic acid) was used with magnetic exposure. Gene expression in magnetic-chemical group was done in day 7 and 10 after addition of osteogenic medium. Expression of osteoblastic marker genes (OCN and Runx2) was evaluated by TaqMan Real-Time PCR. Alizarin Red S staining showed the formed calcium nodules.

**Results:** Real-time results showed the expression levels of Runx2 and OCN were much more increased in magnetic/chemical group in comparison to magnetic and also chemical ones alone.

**Conclusion:** Combination of electromagnetic field and chemical factors is much more suitable for directing osteogenesis and these two elements enhance each other.

**Keywords:** Mesenchymal Stem Cell, Differentiation, Osteogenesis, Electromagnetic Field

Ps-52: A Unique Oct4 Interface and Its Role in Reprogramming to Induced Pluripotency

Jerabek S1, Esch D1, Juha V1, Cojocaru V1, Drexler H1, Araúzo-Bravo M1, Ng C1, Jauch R1, Wilmanns M1, Schöler H1

1. Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany
2. Laboratory for Structural Biochemistry, Genome Institute Singapore, Singapore, Singapore

**Corresponding Email:** stepan.jerabek@mpi-muenster.mpg.de

**Objective:** In 2006, the field of stem cell research was captivated by a possibility of epigenetic reprogramming of terminally differentiated cells to a pluripotent state by four factors - Oct4, Sox2, Klf4 and c-Myc. However, six years after this breakthrough, we still lack proper insight into this fascinating process on a molecular level. Among all four factors in the original cocktail, Oct4 evinces a special feature as it is impossible to replace this transcription factor by any other member of its protein family. But what makes the protein so unique?

**Materials and Methods:** Purification and assembly of the Oct4POU:PORE complex; Crystallization and structure determination; Electrophoretic mobility shift assay; Reprogramming and rescue assay; Streptagged purification of proteins; Mass spectrometry; Yeast two hybrid screen; Genetic code expansion technology.

**Results:** We solved the crystal structure of Oct4. This revealed a unique structured linker sequence of Oct4, which is not found in other POU class members. Moreover, this alpha-helical stretch is exposed to surface, creating an attractive interface regarding direct interactions with other components of the reprogramming machinery. A mutation screen along the linker proved several amino acids to play a crucial role in reprogramming. Based on current interactome studies, we conclude that the region is involved in recruiting key epigenetic players. Two different approaches have been chosen to determine the direct interaction partners of aforesaid Oct4 alpha helix. The results of a yeast two hybrid screen and in vivo crosslinking via the genetic code expansion technology will be presented.

**Conclusion:** The linker region of Oct4 is involved in recruiting key epigenetic players during the reprogramming process.

**Keywords:** Oct4, Reprogramming

Ps-53: Investigations on Amount of Secreted Nitric Oxide from Endothelial Cells in Response to Shear Stress

Kabirian F1,2,3*, Amoabediny G1,2, Zandieh Doulabi B4, Haghighipour N3

1. Life Science Department, Faculty of Interdisciplinary New Sciences and Technologies, University of Tehran, Tehran, Iran
2. Department of Tissue Engineering, Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
3. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
4. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam, Universiteit van Amsterdam and Vrije Universiteit, Amsterdam, The Netherlands

**Corresponding Email:** amoa@ut.ac.ir

**Objective:** Nitric oxide (NO) as an intracellular messenger from the vascular endothelial cells (ECs) plays crucial roles in regulation of regional blood flow by vasodilatation and inhibition of platelet aggregation and adhesion leading to prevention of atherosclerosis and thrombosis. ECs are constantly exposed to blood flow shear stress, which regulate important physiological blood vessel responses such as NO release. The present study aims to address the effects of shear stress on changes in amount of NO secretion from ECs.

**Materials and Methods:** Human Umbilical Vein Endothelial Cells (HUVEC, NCBI Code: C554) were obtained from National Cell Bank of Iran and cultivated in DMEM (Gibco, NY, USA), supplemented with 10% FBS (Gibco, NY, USA) and penicillin/streptomycin (Gibco, NY, USA) at 37°C and 5% CO2 incubator. Silicone tube with 2mm-ID (dedicated from Research Center for New Technologies in Life Science Engineering) were used as scaffold. HUVECs were cultured on the scaffold surface...
modified by collagen type I solution coating. By peristaltic pump and a designed perfusion bioreactor, we applied 1.8 dyne/cm² shear stress to HUVECs and NO concentration were measured at different times in an hour by Griess reagent and read with Elisa reader.

Results: HUVECs released basal level of NO at static (control) condition. At all times NO amount was more than control condition at the same time. Applying 1.8 dyne/cm² shear stress caused a rapid 7-fold increase in NO response of the cells at first 5 minute in an hour compared with control group (p<0.05).

Conclusion: The results showed that HUVECs respond to flow shear stress by release of more NO compared with static condition. Results of this study suggest that applying the shear stress to tissue engineered blood vessels can decrease the possibility of thrombosis through increasing the NO release.

Keywords: Shear Stress, Endothelial Cell, Nitric Oxide

Ps-54: Investigation on Amount of Secreted Nitric Oxide from Endothelial Cells by Applying Shear Stress and Different Temperature

Kabirian F1, 2, 3*, Amoabediny Gh1, 2, Zandieh Doulabi B1, Haghighipour N1

1. Life Science Department, Faculty of Interdisciplinary New Sciences and Technologies, University of Tehran, Tehran, Iran
2. Department of Tissue Engineering, Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
3. Tissue Engineering Laboratory, National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
4. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam-Universiteit van Amsterdam, Vrije Universiteit, Amsterdam, Netherlands

Corresponding Email: amoabediny@ut.ac.ir

Objective: Endothelial cells (ECs), the inner layer of vascular wall, are constantly exposed to blood flow shear stress, which regulate important physiological blood vessel responses such as nitric oxide (NO) release. NO releases in response to the shocks such as shear stress and have some beneficial influence such as anti-thrombotic effect. This article aims to study the effects of temperature stress and combination of shear stress and temperature stress on ECs.

Materials and Methods: By designing a perfusion bioreactor we applied shear stress to ECs cultured on the lumen of tubular silicone scaffold. We applied 25, 30, and 37°C and combination of these temperatures and shear stress to cells to study the amount of NO secretion.

Results: Increase in shear stress and decrease in temperature leads to more increase in NO production than shear stress alone by folds of NO (p<0.05).

Conclusion: NO release by applying the shear stress is more than applying temperature alone. But our results show that to have the most NO release from ECs, applying 1.8 dyne/cm² at 25°C is useful.

Keywords: Endothelial Cells, Shear Stress, Nitric Oxide, Temperature

Ps-55: Effects of Ectopic Expression of Rho\Rock Signaling Cascade Elements on PPARγ Expression

Kajabadi E1, 2*, Ghoochani A2, Ghaedi K1, 2, Peymani M2, 3, Nasr-Esfahani MH2, Baharvand H4

1. Biology Department, School of Sciences, Isfahan University, Isfahan, Iran
2. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Biology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
4. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Corresponding Emails: kamranghaedi@royaninstitute.org and mh_nasr@royaninstitute.org

Objective: Human embryonic stemcells, unlike mouse embryonic stem cells, are vulnerable to cell death upon dissociation. This apoptosis is due to the activation of Rho/Rhock signaling pathway, which results in cytoskeletal phosphorylation due to myosin activation. E-cadherin is one of cytoskeleton elements that will be decreased after Rho/Rhock pathway activation. In this study we evaluate the effects of hESCs dissociation and so Rho/Rhock pathway activation on PPARγ expression in comparison with vital genes involved in colony formation like E-cadherin.

Materials and Methods: Expression level analysis of PPARγ after dissociating hESCs was performed by Real times PCR. To assess whether Rho/Rhock pathway effects on PPARγ is direct or not, co-transfection of two elements of thispathway (RhoA and PIP5K)withPPARγ was performed in CHO cell line and PPARγ expression wasanalyzed in RNA and protein levels by Real times PCR and western blotting.

Results: PPARγ expression was reduced in dissociated hESCs the same as E-cadherin. Rho/Rock signaling cascade elements have direct effect on reducing of PPARγ expression.

Conclusion: Our results showed that PPARγ, the same as vital genes involving in cytoskeletal structures, is directly vulnerable to Rho/Rock signaling pathway activation, which is due to dissociating hESCs.

Keywords: Rho/Rock Signaling Pathway, PPARγ, Human Embryonic Stem Cells

Ps-56: The Effects of Bone Morphogenetic Protein-4 on Cardiomyocyte Differentiation of Mouse Adipose Tissue-Derived Stem Cells

Khaleghi M*, Fakhri Taha M, Javeri A, Jafarzadeh N

Nano BioMaterials and Tissue Engineering Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Corresponding Email: mftaha@nigeb.ac.ir

Objective: The aim of the present study was to evaluate
the effects of BMP-4 on cardiomyocyte differentiation from mouse adipose tissue-derived stem cells (ADSCs).

**Materials and Methods:** ADSCs were isolated from inguinal adipose tissue of 8 to 10-week-old mice. Third-passage ADSCs were cultured to reach 100% confluence. Cardiac differentiation was initiated in DMEM plus 10% fetal bovine serum (FBS) or 15% Knockout Serum Replacement (KoSR). BMP-4 with the final concentrations of 10, 20 or 50 ng/ml was added to the experimental setting for 5 days. After this initial stage, differentiation was continued in FBS-containing medium, and cardiac differentiation was examined after 21 days. Optimization of the differentiation by insulin-transferrin-selenium (ITS) and 5-azacytidine was also investigated. The expression of cardiac transcription factors and cardiac-specific genes was evaluated by RT-PCR. MLC2V and MLC2A mRNAs were compared between groups by quantitative real-time PCR. Moreover, the presence of cardiac-specific proteins was assessed by immunocytochemistry.

**Results:** Three-week differentiated ADSCs showed the expression of cardiac transcription factors, GATA4 and MEF2C, and cardiac-specific genes, α-MHC, β-MHC, MLC2V, MLC2A and ANP. MLC2V mRNA had its highest expression when the initial phase of differentiation was performed in KoSR-containing DMEM and at the presence of 50 ng/ml BMP-4. The highest expression of MLC2A mRNA was detected at the presence of KoSR and 10 ng/ml BMP-4. Supplementing the culture medium of 10 ng/ml BMP-4 group with ITS upregulated the expression of MLC2A mRNA, while neither ITS nor ITS plus 5-azacytidine could increase the expression of MLC2V. Immunocytochemical analyses showed the expression of α-actinin and cardiac troponin I proteins in the ADSC-derived cardiomyocytes.

**Conclusion:** The results of the present study are indicating an important role for BMP-4 in cardiomyocyte differentiation from ADSCs. Moreover, cardiac differentiation was more effective when the initial phase of differentiation was performed in KoSR-containing media.

**Keywords:** BMP-4, Adipose Tissue-Derived Stem Cells, Differentiation, Cardiomyocyte

**Ps-57: Expression of C3(ETF3) in Co-Culture of Ovis Arises Embryos with Mesenchymal Stem Cells**

**Khatami S1*, Ayat H1, Mirshokrae P2, Ahadi AM1**

1. Department of Genetics, Faculty of Science, Shahrekord University, Shahrekord, Iran
2. Department of Reproduction, Faculty of Veterinary science, Shahrekord University, Shahrekord, Iran

**Corresponding Email:** ahadi_al@sci.sku.ac.ir

**Objective:** Co-culture reduces the fragmentation rate of human embryos are cultured in vitro and increases the blastulation rate. The oviduct-derived embryotrophic factors (ETFs) affected the development of embryos. ETF-3 is most abundant among the three ETFs. This factor is a mixture containing complement protein 3 (C3), and its derivatives, C3b and iC3b. Mesenchymal stem cells (MSCs) could improve meiotic maturation in vitro and subsequent embryo developmental potential by secretion of various growth factors and several components. In this study with this assumption that co-culture enhances the vitality of the embryo at blastocyst stage, we use MSCs in culture medium of embryo and detect expression of C3 (ETF3).

**Materials and Methods:** Five groups’ cells in three clusters (mesenchymal stem cell, ovis embryo in 8- to 16-cell and co-culture of Mesenchymal stem cell and embryo cells) were analyzed. Total RNA was extracted using Trizol reagent according modified manual instructed in kit. RT-PCR was used to confirm the expression of C3 (ETF3). All amplified fragments were analyzed by electrophoresis on a 6% polyacrylamide gel and sequenced to verify PCR-products.

**Results:** The data of this study was very significant. Although there was expression in three groups of cells and results were demonstrated via Sequencing, but it must be mentioned, we could use stem cell as a source of production. On the other hand, if the embryo could not express this factor, using of these cells was helpful. Also the other possibility is that the post-translational mechanisms and intercellular interaction regulate using of ETF3 by embryo.

**Conclusion:** Co-culture of embryo with MSCs can improve vitality of embryo in a gene expression manner. The detailed influence of MSCs on blastulation rate and subsequent embryo developmental await further investigation.

**Keywords:** Embryotrophic Factors, Complement Protein 3, Mesenchymal Stem Cells, Co-Culture

**Ps-58: Isolation of A Stably Transformed PC12 Cell Line Expressing Human ANXA1 cDNA**

**Kiani-Esfahani A1,2*, Kazemi S3, Hashemi M1, Peymani M4, Ghaedi K1,3, Nasr Esfahani MH1**

1. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Biology Department, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
3. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran

**Corresponding Email:** kianiroyan@yahoo.com

**Objective:** Parkinson’s disease (PD) is known as a degenerative disorder of the central nervous system. In this disease, the death of dopaminergic neurons in the substantia nigra, a region of the midbrain is occurred. Annexin A1 (ANXA1) is reported as an anti-inflammatory factor. ANXA1 might play a neuroprotective role in PD by acting as an anti-inflammatory mediator. The aim of this study was isolation of PC12 cell line overexpressing ANXA1.

**Materials and Methods:** At first step, cDNA of ANXA1 was prepared from neutrophils and then inserted to PGL268 vector to produce recombinant PGL/ANXA1 vector. The accuracy of cloning was confirmed by further sequencing. Transfection was carried out in the dopamin-
ergic neuronal cell line, PC12 (a cell line derived from a pheochromocytoma of the rat adrenal medulla), using of lipofectamin LTX and after 48 hours EGFP reporter was detected. Furthermore, PC12 cells expressing ANXA1 gene were selected followed by G418 treatment after 2 weeks. Expression of ANXA1 was evaluated by real time PCR, western blot and flow cytometry techniques.

Results: The results indicated strong expression of ANXA1 in PC12 cells.

Conclusion: PC12 cells containing ANXA1 DNA could be further used as an in vitro model for evaluation of ANXA1 Anti-inflammatory effects.

Keywords: Parkinson's Disease, Annexin A1, PC12, Neuroprotective

Ps-59: Comparative Proteomic Analysis of Human Somatic Cells, Induced Pluripotent Stem Cells, and Embryonic Stem Cells

Kim SY1,2*, Kim MJ1, Son MJ1, Han YM2, Lee SCH1, Cho YS3, Bae KH1

1. Medical Proteomics Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea
2. Department of Biological Sciences, KAIST, Daejeon, South Korea
3. Development and Differentiation Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea

Corresponding Email: khbae@kribb.re.kr

Objective: The purpose of this study was to perform a detailed analysis of the proteomes of somatic donor cells, human iPSCs (hiPSCs) derived from the corresponding somatic cells, and human ESCs (hESCs) to validate the usefulness of hiPSCs at the proteomic level.

Materials and Methods: Generation of hiPSCs, Maintenance of hiPSCs and hESCs, Polymerase chain reaction analysis, Immunocytochemistry, Karyotype analysis, Isoelectric focusing and 2-D gel electrophoresis, Staining and image analysis, In-gel digestion and identification by liquid chromatography-MS/MS, Target validation using western blot analysis.

Results: Generation of hiPSCs, Proteomic analysis of donor cells, hiPSCs, and hESCs by 2-DE, Identification and classification of differentially expressed proteins by liquid chromatography-MS/MS, Validation of proteins by western blot analysis, Protein network analysis of identified proteins.

Conclusion: We performed a comparative proteome analysis of hESCs, hiPSCs, and their corresponding donor cells (hFFs). Through this approach, we identified many proteins that may be directly or indirectly involved in reprogramming. The identified proteins are involved in various biological processes, including transcription co-factor activity, proteasome activator activity, lipid metabolic processes, cell redox homeostasis, and nucleoside metabolic processes, indicating that significant physiological changes occur during reprogramming. Further, we identified several proteins with differential expression patterns between hESCs and hiPSCs. In future studies, we will perform a detailed investigation of the roles of the identified proteins during reprogramming and examine whether they can be effectively utilized to induce or regulate reprogramming at will. In combination with our proteomic analyses, further characterization of these proteins should provide valuable new insights into the mechanism of reprogramming.

Keywords: Proteomics, Stem Cell

Ps-60: Peripheral Tolerance Allows Transdifferentiation of Allogenic Bone Marrow Cells into Liver Parenchyma/Mesenchyma Cells for Phenotype Correction of Hemophilia A Mouse

Kochat V1, Kanjirakuzhiyil S1, Baligar P1, Yadav N1, Nagarajan P1, Mukhopadhyay A1*

1. Stem Cell Biology, National Institute of Immunology, National Institute of Immunology, New Delhi, India
2. Small Animal Facility, National Institute of Immunology, National Institute of Immunology, New Delhi, India

Corresponding Email: ashok@nii.ac.in

Objective: Earlier, we have shown that the severity of bleeding disorder can be ameliorated by transplanting uncommitted bone marrow (BM) cells in perturbed liver of hemophilia A (HA) mouse. Since hemophilia is an X-chromosome-linked recessive bleeding disorder, the same can not be treated using autologous BM cells. Transplantation of allogenic cells leads to graft rejection and in worst situation may cause host disease. These immune reactions are commonly suppressed by using immunosuppressive drugs. There are numerous side effects on recipient for using immunosuppressive drugs, the most significant are opportunistic infections and transplant-related malignancies. Therefore, it is necessary to develop an alternate method for immune-suppression, which has no or less side effect on the host. Here, we present an immunological method for development of donor MHC-specific peripheral tolerance that allows engraftment and transdifferentiation of allogenic cells in hemophilic mouse liver.

Materials and Methods: Uncommitted (Lin-) eGFP-expressing BM cells of FVB/J mouse (H2Kq) were co-transplanted with allo-antigen sensitised recipient HA mouse (H2Kb) T-regulatory (Treg) cells. The mice were sacrificed at different times of transplantation for examining effectors CD4+ T cells and CD4+CD25+Foxp3+ Treg cells population in the spleen and delayed-type hypersensitivity (DTH) reactions in the liver. The presence of donor specific cells in the recipient liver and their phenotypes were examined by immunohistochemical analyses. The phenotype correction of HA mice was assessed by quantifying the clotting factor in plasma and tail-clip challenge experiment.

Results: The results suggest that allo-antigen specific Treg cells were able to control the immune reaction by suppressing CD4+ T cells, which was correlated with...
the pathology of the liver tissue. We have also evaluated different cellular sources of FVIII synthesis to find out a potential candidate for treatment of hemophilia A. The results suggest that in addition to hepatocytes, BM-derived sinusoidal endothelial and Kuffer cells expressed FVIII. The transplanted HA mice showed FVIII activity in plasma and survived tail-clip challenge experiment.

**Conclusion:** The allogenic BM-derived hepatocytes, endothelial and Kuffer cells can synthesize FVIII in liver and involved in correcting bleeding phenotype of HA mice. This conversion is possible by inducing peripheral tolerance against allo-antigen.

**Keywords:** Bone Marrow-Derived Cells, Hemophilia A, Treg Cells, Transdifferentiation

Ps-61: Muscle-Fat Interaction and Its Role in Muscle Regeneration and Obesity

**Kuang Sh1,2*, Shan T1, Liu W1, Liang X1, Bi P1**

1. Department of Animal Sciences, Purdue University, West Lafayette, USA
2. Center for Cancer Research, Purdue University, West Lafayette, USA

**Corresponding Email:** skuang@purdue.edu

**Objective:** Obesity and aging both involve ectopic accumulation of adipose (fat) and deterioration of muscle function. Postnatal growth and hypertrophy of skeletal muscle and adipose tissues are maintained by tissue resident stem cells. Adipose stem cells are found in various tissues while muscle stem cells (satellite cells) reside only in the skeletal muscles. Understanding how muscle and fat interact has important implications in the treatment of aging related muscular diseases and obesity.

**Materials and Methods:** Stem cell lineage tracing and lineage ablation analysis, tissue regeneration assay and metabolic analysis in mice are employed to dissect the *in vivo* interaction between muscle and fat and its implication in muscle regeneration and obesity.

**Results:** We discovered that intramuscular adipose progenitors are necessary for proper regeneration of injured skeletal muscles (Liu et al 2012, Dev Biol). By contrast, we show that skeletal muscle cells robustly inhibit the differentiation of adipose stem cells. This is the first study establishing a requirement for adipose tissue in skeletal muscle function and challenges the traditional view that muscle degeneration in the aging population is triggered by ectopic fat accumulation. Instead, our result suggests that ectopic fat accumulation in the aged and dystrophic muscle is a consequence of muscle deterioration (wasting) that abrogates the inhibitory effect of healthy muscle on adipose differentiation. Consistent with this notion, we demonstrate that muscle hypertrophy due to myostatin mutation reduces intramuscular fat content and promotes the conversion of the lipid-storing white adipocyte to lipid-catabolizing brown adipocytes (Shan et al, 2013 FASEB J). We further identify irisin as a myokine (muscle hormone) that mediates the effect of muscle hypertrophy on adipose tissues.

**Conclusion:** Together, our studies demonstrate active interactions between skeletal muscle (stem cells) and adipose (stem cells). We provide compelling evidence that intramuscular adipose is necessary for proper muscle function but muscle hypertrophy promotes the conversion of white adipocytes to brown adipocytes, therefore reducing obesity risks.

**Keywords:** Skeletal Muscle, Adipose, Stem Cells, Regeneration, Muscular Dystrophy, Obesity

Ps-62: The Importance of Design and Heterologous Expression of Anti-Cancer Stem Cells Peptides As A Novel Paradigm for Cancer Treatment

**Latifi Navid H*, Latifi Navid S, Zahri S**

Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, Iran

**Corresponding Email:** hh_latfinavid@yahoo.com

Tissue stem cells form a small population of cells (usually much less than 1% within a given tissue), have self-renewing potential and display significant plasticity. The capacity of stem cells for tissue renewal and damage repair has great potential applications for biotechnology, regenerative medicine and tissue engineering. However, they can pose a serious threat to the host organism if their differentiation potential becomes uncontrolled. Mutations within regulatory pathways can impair stem cell functions so that their high proliferative potential, and frequently their migratory capabilities as well, make these mutated stem cells tumorigenic. These tumorigenic stem/progenitor cell populations are collectively named CSCs. Anticancer drugs kill cancer cells and cause a reduction in tumor mass. Frequently, however, the cancer re-emerges after a few months or even years because the therapy failed to kill the CSCs. Cancer treatments that target CSC through specific markers or signaling pathways critically involved in CSC function could potentially increase the efficacy of current forms of therapy, by reducing the risk of relapse and dissemination. A6 is an 8-amino acid peptide that has been shown to have anti-invasive, anti-migratory, and anti-angiogenic activities. This peptide shares sequence homology with CD44, an adhesion receptor involved in metastasis that is also a marker of cancer stem cells, potentiated the CD44-dependent adhesion of cancer cells to hyaluronic acid and activated CD44-mediated signaling. It seems that the designing peptides with strong stability followed by their heterologous (under vector) expression will provide a clear perspective in cancer treatment.

**Keywords:** Cancer Stem Cells, CD44, A6 Peptide, Heterologous Expression

Ps-63: Comparison of The Growth Curves of Mesenchymal Stem Cells Isolated from Third Molar and First Premolar Human Dental Pulp

**Mahdiyar P1, Tamadon AD1*, Zare Sh2, Robati R1, Tor-**
abi K, Tamadon A, Mehrabani D
1. Biology Department, Science Faculty, Science and Research Branch, Islamic Azad University, Shiraz, Iran
2. Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Fixed Prosthodontics, Faculty of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Corresponding Email: smashayekhan@gmail.com

Objective: Based on previous researches, dental pulp stem cells (DPSCs) were primarily derived from the pulp tissues of permanent third molar teeth. Their embryonic origin, from neural crests, explains their multipotency. These cells are easily accessible with limited morbidity after collection. This study was undertaken to compare the growth curves of mesenchymal stem cells isolated from third molar and first premolar human dental pulp.

Materials and Methods: To obtain DPSCs, pulp tissues were isolated from human third molar and first premolar teeth. They were digested by treating with collagenase type I. The extracted cells were passed from primary culture up to passage 8. To enumerate the cells, the specified number of the cells was seeded into 24-well culture plates and the number of colonies was counted to determine the growth curves of isolated cells from both types of teeth.

Results: The human DPSCs from both third molar and first premolar teeth were spindle-shaped in morphology. As growth curves show, the proliferation rate of DPSCs in 8th passage among both teeth was different denoting to an increase in doubling time in 3rd molar when compared to first premolar teeth.

Conclusion: DPSCs can be an attractive candidate in regenerative medicine. As growth curves show, 3rd molar tooth is suggested as a better source of MSC isolation.

Keywords: Growth Curve, Mesenchymal Stem Cell, Molar Tooth, Premolar Tooth, Dental Pulp

Ps-64: Differentiation of Mesenchymal Stem Cells to Smooth Muscle Cells on Nanostructured Scaffold for Bladder Tissue Engineering

Mashayekhan S, Moghadasi Boroujeni S, Vakilian S, Yaghmai S

Department of Chemical Engineering, Sharif University of Technology, Tehran, Iran

Corresponding Email: smashayekhan@gmail.com

Objective: Severe bladder dysfunction can be induced by disease or surgical intervention altering the normal pattern of storage and voiding. Severe cases of bladder failure typically do not respond to the most conservative treatment. Currently, the treatment of choice in these patients is an enterocystoplasty, a surgical enlargement of the bladder using intestinal tissue. The primary aim of the surgical reconstruction is to increase bladder capacity and compliance. This method fails to restore emptying function. Furthermore, enterocystoplasty is associated with numerous complications such as metabolic disturbance, increased mucus production, infections and even malignant diseases. Tissue engineering, using autologous cells for implantation might offer a solution to this problem. Recently, several studies have confirmed feasibility of bladder reconstruction using engineered segments which were formed using bioMaterials seeded with autologous cells in vitro. Mesenchymal stem cells (MSCs) have potential to differentiate into all three germ layers and compared with embryonic stem cells demonstrate considerable advantages, including stable differentiation into specific cell lineages, no transdifferentiation into a malignant phenotype, no requirement for the sacrifice of human embryos for their isolation and no or little immune rejection.

Materials and Methods: In this study, the hybrid nanofibrous scaffold of polycaprolactone/polyactic acid was synthesized by electrospinning method. MSCs were seeded on the prepared nanostructured scaffold. Transforming growth factor beta1(Tgf-β1) and ascorbic acid were added to the culture medium in order to induce differentiation of MSCs to smooth muscle cells (SMCs).

Results: Adhesion and proliferation of the cells were successfully demonstrated and finally the potential of the differentiation of MSCs to SMCs were investigated.

Conclusion: Our results demonstrated that the cultured MSCs on the nanostructured scaffold possess the capability of differentiation towards SMCs, showing the potential use of the prepared scaffold in bladder tissue engineering.

Keywords: Mesenchymal Stem Cells, Bladder Tissue Engineering, Nanofibrous Scaffold

Ps-65: Expansion of Mesenchymal Stem Cells on Porous Chitosan-Gelatin Microcarrier in Suspension Culture

Mashayekhan S, Karimian Shamsabadi M, Yaghmai S

Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran

Corresponding Email: mashayekhan@sharif.edu

Objective: Mesenchymal stem cells (MSCs) have potential clinical utility in the treatment of a multitude of ailments and diseases, due to their relative ease of isolation from patients and their capacity to form many cell types. However, MSCs are sparse, and can only be isolated in very small quantities, thereby hindering the development of clinical therapies. Furthermore, because of the specific properties of these cell populations, there is a need to investigate novel cell culture strategies adapted from established dynamic cultivation practices. The use of microcarrier-based stirred suspension cultures to expand stem cell populations offers an appropriate approach to overcome this problem.

Materials and Methods: In the current study porous chitosan-gelatin microcarriers were successfully fabri-
cated for use in cultivation of stem cells. A modified syringe pump method followed by freeze-drying was used for fabricating the porous chitosan-gelatin microcarrier beads. Various factors that influenced the preparation of microcarriers were studied and the conditions were optimized to prepare microcarriers having the desired size, porosity, mechanical elastisity and biocompatibility. MSCs were loaded on the microcarriers with standard culture protocol commonly reported in the literature in a 48-well plate and then the plate put onto a mini-rocker to facilitate expansion of MSCs.

Results: The behavior (attachment, expansion and morphology) of the cells on the microcarriers were investigated in order to check the suitability of the prepared microcarriers for stem cell cultivation. The viability of immobilized cells on the microcarriers was investigated by an MTT assay.

Conclusion: The observation results showed that cellular morphology was homogenous around the microcarriers and MSC’s expanded gradually on the microcarriers surface.

Keywords: Mesenchymal Stem Cells, Microcarriers, Expansion, Suspension Culture

Ps-66: Comparison of The Chromosomal Stability of Mouse Embryonic Stem Cell in Medium Containing R2i (TGF-ß and ERK1,2 Inhibitors) with Medium Containing 2i (GSK-3 and ERK1,2 inhibitors) by Karyotyping

Masoudi NS*, Mehrabi HK, Hassani SN, Mohseni Meibodi A, Mollamohamadi S, Zarei Moradi Sh, Mansouri Z3, Gourabi H

1. Department of Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Genetics at Reproductive Biomedicine Research Center, Ahar Branch, Islamic Azad University, Saveh, Iran

Corresponding Email: gourabi@royaninstitute.org

Objective: Mouse embryonic stem cells are pluripotent cells which are derived from the blastocyst cells of the preimplantation embryos. The success rate in generating embryonic stem cells in some mouse strains like BALB/c, C57BL/6, DBA/2 has been very low and altered by the genetic variations. Nowadays the researchers have succeeded in producing embryonic stems cells from these resistant strains by identifying the cell signaling pathways involved in pluripotency maintenance and using differentiation inhibitory small molecules. In this study the chromosomal integrity of mouse embryonic stem cells after exposure to two different mediums containing ERK1,2 and GSK3 pathways inhibitors (2i) and ERK1,2 and TGFβ pathways inhibitors (R2i) were investigated.

Materials and Methods: 9 cell lines were derivated from BALB/c, C57BL/6 and DBA/2 mouse strains (3 cell lines from each strain). These lines were cultured in R2i medium and after 10 passages, were studied for chromosome stability by G-band karyotype analysis. The normal karyotype lines were selected and divided to two groups, each were cultured in 2i and R2i medium for 20 passages. Then these two groups were karyotyped to evaluate their chromosome integrity.

Results: The rate of numerical and structural abnormalities in the proliferated cells in 2i medium were significantly higher than that of the cells on the R2i medium (in comparison with the primary 10 passages in R2i medium). The mean of chromosomal abnormalities of all proliferated cells in 2i medium were significantly higher than the cells in R2i medium (39.33 and 22.66% respectively). It should be mentioned that the BALB/c strain showed the least chromosomal abnormality percentage, while the DBA/2 strain had the most abnormality.

Conclusion: Inhibiting GSK3 pathway during cell divisions in mouse embryonic stem cells may have more destructive effect on chromosomal integrity than inhibiting TGFβ.

Keywords: Small Molecules, GSK3, ERK1,2, TGFβ

Ps-67: In Vitro Fertilization Rate Improvement and Decrease of Apoptosis Incidence in Rams Following Feeding with Omega-3 Source

Matini A**, Ebrahimi B¹, Alizadeh AR¹, Dalman A², Rashki L²

1. Department of Animal Science, Saveh Branch, Islamic Azad University, Saveh, Iran
2. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Corresponding Email: bt.ebrahimi@royaninstitute.org

Objective: Omega-3 is found in high levels in mammalian spermatozoa and it could improve sperm classical parameters that correlate closely with fertilization. The aim of this study was to evaluate the apoptosis incidence and in vitro fertilization (IVF) rate following ram diets supplementation with omega-3 source.

Materials and Methods: Eight of 2-3 years old Zandi rams (live weight: 65 ± 10kg) was randomly assigned into two groups: control (CTR) and fish oil (FO). Energy, protein and vitamin E supplement level was similar in both groups. Rams in FO group received maintenance diet in association with 35gr/day/ram fish oil. diet were fed for 70 days. After adaptation period (21 d), semen was collected from each ram by artificial vagina and transferred to laboratory. Fertilization capacity of swim-up processed semen was only assessed at the first and last day of sampling by in vitro fertilization technique, but apoptosis incidence was evaluated in every week of sampling by flow cytometry technique.

Results: Flow cytometry results showed that totally the occurrence of apoptosis in the FO group was lower than CTR group. This difference was only significant in the 3rd week of sampling (p<0.05). Also two cell stage embryos rate following IVF in two groups indicated signifi-
Ps-67: Angiotensin- Converting Enzyme Inhibitors Have Beneficial Effects on Endothelial Cell Dysfunction

Meamar R, Dehghani L
t, Ghasemi M

Department of Neurology, Isfahan Neurosciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
Corresponding Email: meamar@pharm.mui.ac.ir

Objective: Alzheimer's disease (AD) is a progressive neurodegenerative disease which endothelial cell can be affected by this disease. In brain, functional changes in endothelial cells contribute to reductions in resting blood flow and following progression of neurologic disorders. Furthermore, Angiotensin-converting enzyme inhibitors (ACE-I) have beneficial effects on endothelial dysfunction. This is the first study which presents direct experimental evidence associating to endothelial apoptosis as a basis of AD pathogenesis and response to an ACE-I therapy.

Materials and Methods: Human umbilical vein endothelial cells (HUVECs) were treated with sera from AD patients and sera from healthy volunteers (each n = 10). Apoptosis was determined by annexin V-propidium iodide staining and cell death detection kit. The effect of 50μM Enalapril as a ACE-I drug on EC apoptosis was assessed. Nitrite (NO2-) levels were determined in the culture supernatants.

Results: Enalapril suppressed the induction of apoptosis by the serum of patients only when used before treating HUVECs with the sera of AD. Mean ± SD of apoptosis induction in the control group was 6.7 ± 3.69; in the group treated with sera of AD for 24 hours was 47.78 ± 0.65; in the group wherein sera from AD was added (pretreatment) after exposure of HUVECs by 50 μM enalapril for 24 hours was 26.6 ± 2.63; and in the group where in HUVECs were exposed in the sera of AD for 24 hours and then 50μM enalapril was added to these cells for another 24 hours (post treatment) was 56.87 ± 5.51. Also, the mean ± SD of NO2 concentration showed significantly greater levels of dissolved NO2/NO3 metabolite in the culture media of untreated HUVECs by enalapril (1.03 ± 0.06) as compared with control (0.26 ± 0.13; p < 0.05), while the rate of nitric oxide (NO) significantly decreased when enalapril was presented in culture both in the pre-treatment (0.07 ± 0.003) and in the post treatment group (0.06 ± 0.005; p < 0.05).

Conclusion: It could be concluded that EC treated with sera from AD patients activates apoptosis in HUVECs; this effect was reversed by enalapril pretreatment. This can be proposed as a therapeutic approach for Alzheimer’s patients.

Keywords: ACE-I, Endothelial Cell, Apoptosis

Ps-68: Effects of Ecstasy in Self-Renewal Properties Mediated with Glutamate Receptor in Mouse Embryonic Stem Cells

Meamar R1, 2, Dehghani L1, 2*, Karamali F1, Baharvand H1, Nasr-Esfahani MH1

1. Department of Reproductive Biotechnology at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Tehran, Iran
2. Isfahan Neuroscience Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Corresponding Email: meamar@pharm.mui.ac.ir

Objective: Ecstasy or methylenedioxymethamphetamine (MDMA) is a potent psychomimetic drug. It is well established that this compound is neurotoxic both in vivo and in vitro. One of the purposed mechanisms for this toxicity is a secondary release of glutamate. Glutamate induces its neurotoxicity through mGlu5 receptors. In recent decades embryonic stem cell lines were used to examine pharmacological and toxicological effects of drugs in vitro and it has been reported that mGlu5 is the only glutamate receptor expressed in mESCs (mouse embryonic stem cells) and plays a role in maintenance and self-renewal properties of mESCs. In this study aims to investigate whether ecstasy could influence self-renewal via the mGlu5 receptor in mESCs.

Materials and Methods: In this experimental study, we used immunocytochemistry and reverse transcription-polymerase chain reaction (RT-PCR) to determine the presence of the mGlu5 receptor in mESCs. The stemness characteristic in treated mESCs by immunofluorescence and flow cytometry was studied. One-way ANOVA or repeated measure of ANOVA according to the experimental design was used for statistical analyses.

Results: In this study mGlu5 expression was shown in mESCs. ecstasy (450 μM) induced a significant increment in self-renewal properties in mESCs but did not reverse 2-methyl-6(phenylethynyl) pyridine (MPEP, 1 μM), a non-competitive selective mGlu5 antagonist.

Conclusion: We reported a binary role for ecstasy on mESCs; it reduced mESCs proliferation while maintaining its self-renewal. Pharmacological blockade of the mGlu5 receptor with MPEP before addition of ecstasy only partially reduced this effect. This suggests that ecstasy mediated its role through a different mechanism, which requires further investigation. In conclusion, despite being toxic, ecstasy maintains stemness characteristics.

Keywords: Embryonic Stem Cells, Ecstasy, Self-Renewal, Glutamate Receptor
Ps-70: Experimental Validation of A Novel Alternative Polyadenylation Isoform of Human spp1 Gene in U87-MG Cell Line

Mehrarav M*, Najafi H, Mohamad Soltani B

Department of Molecular Genetics, Faculty of Biology Sciences, Tarbiat Modares University, Tehran, Iran
Corresponding Email: majid_gene@yahoo.com

Objective: Osteopontin (OPN), also known as SPP1 (secreted phosphoprotein) is a secreted protein present in bodily fluids and tissues. It is also a tumour-associated protein, and elevated OPN levels are associated with tumour formation, progression and metastasis. In cancer patients expression of OPN has been associated with poor prognosis in several tumor types including breast, lung, and colorectal cancers. Research has revealed a promising role for OPN as a cancer biomarker. OPN is subject to alternative splicing, as well as post-translational modifications such as phosphorylation, glycosylation and proteolytic cleavage. Functional differences have been revealed for different isoforms and post-translational modifications. The pattern of isoform expression and post-translational modification is cell-type specific and may influence the potential role of OPN in malignancy and as a cancer biomarker. Tumor specific splice variants are being discovered at an increasing rate and their functions are also investigated in cancer progression.

Materials and Methods: After culturing U87-MG cell line in proper medium, RNA extraction following by single strand cDNA synthesis has been performed. Multiple products of RT-PCR by specific primers for spp1 mRNA was isolated and cloned in pTZ57R/T vector for sequencing. We found a novel transcription variant of spp1 gene in U87-MG cell line, experimentally. Our sequencing data revealed that this novel variant lacks a region that potentially is a binding site of some micro-RNAs.

Conclusion: Our study revealed that this novel variant has same function with other variants of this gene but we assume that post-transcriptional control mechanisms for this variant are different from the other variants. Computational analysis reveals that shorter variant of spp1 gene has so many regions that are complementary with some micro-RNAs. Then shorter variant is more stable than longer one and has a significant association with cancer.

Keywords: Spp1 Gene, ONP, Transcription Variant, U87-MG Cell Line

Ps-71: Evaluation of miR-210 Effect on Proliferation and Survival of Mouse Mesenchymal Stem Cells

Minayi N*, Mohammadian M, Taybei B, Alisadeh Sh

Department of Hematology Allied Medical School, Hematology Lab, Tehran University of Medical Sciences, Tehran, Iran
Corresponding Email: alizadehs@sina.tums.ac.ir

Objective: Bone marrow derived mesenchymal stem cells (MSCs) are a population of multipotent progenitors which have the capacity of proliferation and differentiation into mesenchymal lineage cells. Micro-RNAs are endogenous 22 nt RNAs that can play important roles in some processes such as proliferation and differentiation. We hypothesized that miR-210 could help for better proliferation MSCs since this miRNA can activate HIF pathway. So MSCs could preserve their differentiation ability under normoxic conditions without any growth factors.

Materials and Methods: MSCs isolated from C57 BL/6 mice by flushing its femurs into cell culture media. After 72 hours MSCs which are plastic adherent cells were attached to the flask and nonadherent cells were removed. Subsequently MSCs differentiated into osteocytes and adipocytes with specific differentiation media to confirm their identity and multipotency. Also we were inserted miR-210 in Lentivirus vectors and affected them on MSCs. The expressions of miR-210 and HIF-1α in each passage were evaluated by Real time PCR.

Results: Comparison between miR-210 infected MSCs and control cells showed that miR-210 has ability to increase proliferation of MSCs while maintained their ability to differentiate into adipocytes and osteocytes. The expression of miR-210 and HIF-1α were up regulated in each passage.

Conclusion: In order to important roles of MSCs, proliferation and maintenance of their ability are necessary. We showed that miR-210 has ability to proliferate MSCs without any effect on their differentiation abilities. More studies are needed for evaluation of probably miR-210 effects mechanism on MSCs.

Keywords: Mesenchymal Stem Cells, MiR-210, HIF-1α

Ps-72: Culture and Differentiation of Mouse Neural Stem and Progenitor Cells in A Polymeric Nano-Scaffold Poly-L Lactic Acid

Miri Komak V**, Golmohammadi MG2, Asadi A1, Sagha M2

1. Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabili, Iran
2. Department of Anatomy, School of Medicine, Ardabili University of Medical Sciences, Ardabili, Iran
Corresponding Email: m.golmohammadi@arums.ac.ir

Objective: The selection of a good quality scaffold is an essential strategy for tissue engineering. Ideally, the scaffold should be a functional and structural biomimetic of the native extracellular matrix and support multiple tissue morphogenesis. Neural stem/progenitor cells (NSCs) are intrinsically capable of differentiating into different neural cell types within the nervous system, offering prospects for NSC-based cell therapies to treat neurodegenerative diseases. In this study, an attempt was made to develop porous polymeric Nano-fiber scaffold using a biodegradable Poly (l-lactic acid) (PLLA) for in vitro culture of neural stem and progenitor cells.

Materials and Methods: To fabricate Nano-fiber, we
used electrospinning techniques. The physico-chemical properties of the scaffold were fully characterized by using scanning electron microscopy. NSCs were isolated from the subventricular zone of the adult mouse brain and cultured in the scaffold and then the viability, proliferation and differentiation of these cells determined via both of Immunostaining and MTT assay. Scanning Electron Microscopy (SEM) was also used to observe the morphology of the cells cultured on PLLA fibrous scaffolds.

Results: DAPI and Immunostaining revealed the neuronal differentiation and adhering of cells on the surface of the electrospun scaffolds. Blue stained cells were found in Nano-fiber indicating that the scaffold had provided basic cell attachment. The MTT assay results showed that the absorption in 570 nm increased during 2 to 5 days. The morphology of differentiated cells was evaluated by SEM.

Conclusion: Nano-Scaffold PLLA due to the biocompatible properties is an appropriate structure for proliferation, differentiation and normal growth of the neural stem and progenitor cells.

Keywords: Tissue Engineering, Nano-Fiber Scaffold, Neural Stem Cells

Ps-73: Inside into ID Basic Helix-Loop-Helix Genes Expression during Retinoic Acid Induced Differentiation of Human Embryonic Carcinoma Cells

Moeinvaziri F1,2*, Favaedi R1, Shahhoseini M1

1. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
Corresponding Email: m.shahhosein@royaninstitute.org

Objective: The basic helix-loop-helix (bHLH) family of transcription factors are thought to affect the balance between cell proliferation and differentiation in coordinated regulation of gene expression and cell lineage commitment in most mammalian tissues. Helix-loop-helix ID (Inhibitor of DNA binding) proteins are distinct from bHLH transcription factors in that they do not possess basic domain necessary for DNA binding and function as dominant negative regulators of bHLH transcription factors. It is supposed that various members of the ID family have active roles in embryonic stem cell self-renewal and also in a range of human tumors. Since embryonal carcinoma (EC) cells are malignant but their terminally differentiated derivatives are not, understanding the expression profile of these embryonal cells may be of value for therapeutic purposes in embryology and development. These cells can be differentiate into neural lineage upon exposure to retinoic acid (RA), an analogue of vitamin A, which is a robust inducer during normal mammalian development.

Materials and Methods: In the current work, differentiation was induced by retinoic acid in a human embryonal carcinoma cell line, named NT2/NTERA2. The cells were harvested at 0, 1, and 3 days of RA induction. The mRNA expression levels of all four ID genes were quantitatively evaluated before and after RA treatment by real time-PCR technique (qRT-PCR).

Results: The results declared significant expression levels of ID gene family in embryonal carcinoma cells. During development although onset of differentiation showed increase in the levels of ID genes, the levels of their mRNA decreased by day 3 of differentiation.

Conclusion: Elucidation of the detailed pattern of ID genes expression would reveal novel roles for promoting our understanding of how differentiation and proliferation are regulated.

Keywords: bHLH, ID, NTERA2, Differentiation

Ps-74: Intra-Renal Arterial Injection of Autologous Bone Marrow-Mesenchymal Stem Cells Ameliorates Cisplatin-Induced Acute Kidney Injury in a Rhesus Macaque Mulatta Monkey Model

Moghadasali R1,2*, Azarnia M1, Baharvand H2, Hajinasrola M3, Arghani H3, Nassiri SM4, Molazem M5, Najjarasi M2, Ajdari Z6, Aghdami N7

1. Department of Biology, Kharazmi University, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Urology and Nephrology Research Center, Modares Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
5. Department of Veterinary Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Corresponding Email: rezamoghadasali@yahoo.com

Objective: Acute kidney injury (AKI) is a potentially devastating disease in clinical medicine. However, no specific therapy improves the rate or effectiveness of the repair process after AKI. Bone marrow-derived mesenchymal stem cells (BM-MSCs) have been proven to be benefit to the renal repair after AKI in different experimental models of rodent models, but the consequence of these results to large animals and eventually, to humans remains unknown. Thus, the aim of this study was to assessment the effect of autologous rhesus Macaque monkey BM-MSCs transplantation in cisplatin-induced AKI.

Materials and Methods: BM-MSCs were characterized for their growth characteristics, differentiation potential, immunophenotypic properties and chromosome content. According to design procedure, monkeys were divided into control, vehicle and cell transplantation (Cell Tx) groups and exposed to cisplatin 5mg/kg as intravenous for induce of AKI. Control animals were not treated with anything but cell Tx and vehicle animals were treated with intra-renal arterial injection of autologous BM-MSCs and normal saline, respectively. For cell tracking with magnetic resonance imaging (MRI), BM-MSCs labelled with nanoparticles superparamagnetic iron oxide (SPIO).

Results: Labelled BM-MSCs were found in both glomeruli and tubules. Transplantation of 5×10^5 cell/kg
ameliorated renal function during first week as reflected by significantly lower serum creatinine and urea values and higher urine creatinine and urea clearance without hyponatremia, hyperkalemia, proteinuria and polyuria to 3 months in compare with vehicle and control groups. BM-MSCs markedly accelerated present of Foxp3+ regulatory T (Treg) cells in response to cisplatin-induced damage, as revealed by higher numbers of Foxp3-positive cells within the tubuli with respect to cisplatin-treated monkeys in control and vehicle groups. However, BM-MSCs did not repair kidney parenchyma and as partially to modulate ultrastructure of kidneys.

Conclusion: These data demonstrate that BM-MSCs in this unique cisplatin-induced AKI large-animal model, did exhibit reparative and protective properties.

Keywords: Rhesus Macaques’ Bone Marrow Mesenchymal Stems Cells (rBM-MSCs), Acute Kidney Injury (AKI), Flowcytometry, Histopathology, Immunohistochemistry, Transition Electron Microscopy (TEM), Superparamagnetic Iron Oxide (SPIO), Magnetic Resonance Imaging (MRI)

Ps-75: Preconditioning of Mesenchymal Stem Cells Increases Survival of The Cells: An In Vitro Study

Mohammadi Roushandeh A**, Mortazavi M¹, Habibi Roudkenar M²

1. Anatomical Sciences Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
Corresponding Email: a.mohammadiroshandeh@umsha.ac.ir

Objective: A growing number of experimental studies highlight the potential of stem cell transplantation as a novel therapeutic approach for diseases. This massive loss of stem cells post-engraftment is an impediment that lessens the effectiveness of cell transplantation therapy. Present study tried to increase the survival of the stem cells with H2O2.

Materials and Methods: Mesenchymal stem cells were isolated from rat bone marrow. MSCs from passage four were cultured in DMEM medium containing different concentration of H2O2 and 48 hours and then were recovered with fresh medium. Finally treated cells once were exposed with lethal dose of H2O2 (300μM and 500 μM). The viability of the cells evaluated with trypan blue and proliferation assay with MTT assay.

Results: Preconditioning with 5μM H2O2 significantly increased the resistance of these cells against lethal conditions induced by H2O2 (p<0.05). MTT assay showed higher proliferation in groups with 5 and 10 μM H2O2 after 24 hours.

Conclusion: Preconditioning of MSCs with oxidative stresses enhances their survival, therefore it could increase the efficacy of MSCs transplantation.

Keywords: Hydrogen Peroxide, Preconditioning, Cell Viability, MSCs

Ps-76: The Effect of Cerebrospinal Fluid on Neural Differentiation of Human Umbilical Cord Mesenchymal Stem Cells

Mohammadzade Z*, Farivar Sh

Department of Genetics, Biological Science Faculty, Shahid Beheshti University, Tehran, Iran
Corresponding Email: z.mohamadi2010@gmail.com

Objective: Wharton’s jelly is the gelatinous connective tissue from umbilical cord and is composed of umbilical cord mesenchymal stem cells, collagen fibers, and proteoglycans. The stem cells in WJ of the umbilical cord have properties that make them of interest. For example, they are simple to harvest through noninvasive methods, provide large numbers of cells without risk to the donor, the stem cell population may be expanded in vitro, cryogenically stored, thawed, genetically manipulated and differentiated in vitro.

Materials and Methods: The cells in passage 2 were induced into neural differentiation with different concentration of human Cerebrospinal fluid. Differentiation along the neural lineage was documented by expression of three neural marker Nestin, MAP2 and GFAP for 21 days. The expression of the identified genes was confirmed by Reverse Transciptase PCR.

Results: RT-PCR showed that Cerebrospinal fluid could promote expression of Nestin, MAP2 and GFAP mRNA in an dose-dependant manner, especially at the concentration of 200 μl/ml.

Conclusion: CSF induce neurogenesis of human Wharton’s jelly stem cells, which encourage tissue engineering applications of these cells in neurodegenerative defects and traumatic brain injury.

Keywords: Stem Cells, Umbilical Cord

Ps-77: Mesenchymal Stem Cell Derived Microvesicles: Trophic Shuttles for Enhancement of Sperm Quality

Mokarizadeh A**, Rezvanfar MA², Abdollahi M², ³

1. Department of Immunology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Department of Toxicology, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran
3. Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran
Corresponding Email: arammokarizadeh@yahoo.com

Objective: Diminishing sperm quality during cryopreservation process is the major problem in the field of reproductive biotechnology. The final consequence of such destructive events would be a complete or partial loss of sperm’s fertility. Therefore, rehabilitation of such affected sperms would be a crucial aim to improve sperms quality and fertility. A variety of evidence has indicated that secreted factors from mesenchymal stem cells (MSCs) are involved in regeneration of injured endogenous cells. Recently, MSCs-derived microvesi-
cles (MVs) have been known as potent carriers of such trophic molecules. So, based on the demonstrated roles of these trophic molecules in improvement of sperm’s motility, capacitation and survival, here, we introduce mesenchymal stem cell-derived microvesicles as potent trophic organelles to enhance sperm quality and fertility potential.

**Materials and Methods:** Sperms were obtained from cauda epididymis of 18 weeks old male Wistar rats. Collected sperms from each rat were equally assigned to four separate groups. Following suspension in cryoprotectant extender, sperm were untreated or treated with increasing concentrations of MSC-derived MVs (25, 50 and 100µg). After 3 hours incubation, sperms were cryopreserved. The frozen-thawed sperms were undergone to assess for viability, motility and anti-oxidant capacity parameters. Consequently, the frequency changes of apoptotic and necrotic sperms and expression levels of sperm surface adhesion molecules (CD29, CD44, ICAM-1 and VCAM-1) were assessed by flow cytometry.

**Results:** Results showed the enhanced quality and adhesive properties of cryopreserved sperms following treatment with MSC-derived MVs.

**Conclusion:** Based on the demonstrated beneficial roles of these trophic molecules, here, we introduce mesenchymal stem cell-derived exosomes as potent trophic organelles to enhance sperm quality.

**Keywords:** Cryopreservation, Sperm, Mesenchymal Stem Cell-Derived Microvesicle

**Ps-78: Microvesicles Derived from Mesenchymal Stem Cells: Potent Organelles for Induction of Tolerogenic Signaling**

Mokarizadeh A,1, Delirezh N,2, Morshed A,2, Mosayebi G,2, Farshid AA,1, Mardani K,2, Rezvanfar MA6

1. Department of Immunology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
3. Department of Immunology and Microbiology, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran
4. Department of Pathobiology and Head, Electron Microscope Center, Urmia University, Urmia, Iran
5. Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
6. Department of Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

**Objective:** Generation and maintenance of immunological tolerance is a pivotal aim in the field of autoimmunity. Regulatory molecules of Programmed Death Ligand-1 (PD-L1), Galectin-1 and TGF-β are described as key mediators of peripheral tolerance that actively suppress auto-reactive cells and inhibit their mediated tissue damages. Accordingly, biological intervention in host immune system for induction of peripheral tolerance is pivot to many of the recent studies. Mesenchymal stem cell-derived microvesicles (MVs) are viewed as potential mediators to shed peripheral tolerance toward auto-reactive cells via bearing of tolerogenic molecules.

**Materials and Methods:** Here, MVs were isolated from mesenchymal stem cell (MSC) cultures’ conditioned medium. They were explored for the expression of PD-L1, Galectin-1 and membrane bound TGF-β through flow cytometry. The immunoregulatory effects of MVs on splenic mononuclear cells (MNCs) derived from experimental autoimmune encephalomyelitis (EAE) affected mice were investigated using MTT assay, ELISA and flow cytometry.

**Results:** MVs derived from MSCs expressed PD-L1, galectin-1 and membrane-bound TGF-β. MVs exhibited the potential to inhibit auto-reactive lymphocyte proliferation and also the potency to promote them to secret anti-inflammatory cytokines of IL-10 and TGF-β. Interestingly, inducing inflammatory setting on MSCs, revealed the enhancing regulatory effects of MVs via increased expression of some regulatory molecules, specifically PD-L1 and TGF-β. Induction of tolerogenic signaling, promotion of CD4+ CD25+ Foxp3+ regulatory T cells generation and apoptotic activity towards activated T cells are shown to be possible mechanisms involved in MV-mediated regulation.

**Conclusion:** Recent study suggests MSC-derived MVs as potent organelles for induction of peripheral tolerance and modulation of immune responses.

**Keywords:** PD-L1, Galectin-1, TGF-β, Mesenchymal Stem Cell, Microvesicle, Experimental Autoimmune Encephalomyelitis

**Ps-79: Development of A New Wound Dressing; Mixture of Chitosan Hydrogel (CH) and Amniotic Membrane Extract (AME)**

Momeni M,1*, Zarehaghighi M,2, Ebrahimi M1,2,3, Khorasani GHA3, Hajimiri MH4, Dinavand R5, Shayan Asl N5, Nekookar AH6, Sodeifi N,1, Najar Asl M1

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Surgery, Faculty of Medicine, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Regenerative Biomedicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Pharmaceutics, Faculty of Pharmacy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
5. Animal Core Facility at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Tehran, Iran

**Objective:** Early covering of the burned area is crucial and important factor for successful treatment. Therefore, finding an appropriate Materials for dressing and accelerating wound healing is important. Our previous results determined that amniotic membrane extract (AME) contain many factors that can promote limbal stem cell...
proliferation and corneal epithelialization. In the present study we investigated the effect of mixture of AME and hydrogel based on chitosan on partial burns in Rat model.

Materials and Methods: To find the effective dose of AM extract on skin cells, skin fibroblasts and melanocytes were cultured in 12 well plates for 24, 48 and 72 hours in presence of different concentration of AME (0.1-2 mg/ml). Proliferation rate was measured by manual cell count. In next step, 30 Wistar male rats were anaesthetized and a 5×6 cm partial burn wound was created by 90°C water in 6 second, and then divided in four groups. Group 1: control (no dressing), group 2: chitosan hydrogel (CH) sheet, group 3: CH contains 1mg/ml of AME up to 10th day then followed by CH contains 0.1mg/ml, group 4: CH contains 0.1 mg/ml AME. In Day 10th and 15th, biopsies were taken from wound region for pathological evaluation and Masson’s Trichrome (MT) staining.

Results: Our results determined that 0.1 mg/ml of AME increased fibroblast and melanocyte proliferation significantly (p<0.05), however 2 mg/ml AME Reduced cell proliferation. On the other hand, dressing of wound by hydrogel contains 1mg/ml AME for 10 continues days significantly decreased the wound area six fold compared control group (p<0.01). Pathological experiments showed that collagen fibers increased, inflammatory cells and scar formation decreased in rats which treated with hydrogel contains 0.1mg/ml and 1 mg/ml AME.

Conclusion: According to these results, for the first time we introduce a suitable biological dressing without any side effect that accelerates wound healing.

Keywords: Amniotic Membrane Extract (AME), Hydrogel, Proliferation, Rat, Wound Healing

Ps-80: In Vitro Differentiation of Human Adult Stem Cells into Hepatic Lineage Cells

Montaser L1*, Metwaly H2, El-Bassuoni M1, Tawfeek G3
1. Clinical Pathology, Shebin El-Kom, College of Medicine, Menoufia University, Shebin El-Kom, Egypt
2. Clinical Pathology, Cairo, College of Medicine, Cairo University, Cairo, Egypt

Corresponding Email: lailamontaser@gmail.com

Objective: Liver pathologies affect hundreds of millions of patients worldwide. Millions of patients worldwide suffer from end-stage liver pathologies, whose only curative therapy is liver transplantation. Procedures of liver transplantation are still limited by the donor scarcity, the high costs, and the lifelong immunosuppressive treatments that they all require. Thus, the development of cell therapies for the treatment of end-stage hepatic diseases is currently under investigation all over the world. Adult stem cell (ASC) therapy could solve the problem of degenerative disorders, including liver disease in which organ transplantation is either inappropriate or there is a shortage of organ donors. Objective: Evaluation of the feasibility and efficacy of human bone marrow mesenchymal stem cells (BM- MSCs) to be separated, characterized by the flow cytometer and in vitro differentiated into hepatocyte like cells using different growth factors.

Materials and Methods: 10 ml bone marrow was aspirated from 28 patients. MSCs were identified by their morphology; phenotypic markers were assessed by the flow cytometer using CD73, CD44 and CD34. Isolated MSCs then cultured in differentiating media using different growth factors (b-FGF and HGF) without using extra cellular matrix (ECM). The differentiating ability to hepatocytes was assessed by morphological changes, expression of hepatocyte specific genes (Albumin and Alfa fetoprotein) and also by synthesis & secretion of albumin. The detection of gene expression of Alb and AFP was done by reverse transcriptase polymerase chain reaction (RT-PCR).

Results: The cells isolated from BM by density gradient centrifugation and plastic adherence takes the fibroblast like morphology, adherent to the plastic surface and expresses CD73 and CD44 but not express hematopoietic marker CD34. MSCs cultured in differentiating media changed into hepatocyte like morphology, express AFP, Albumin genes and also secrete albumin in the supernatant of culture. Combination of HGF and b-FGF for hepatogenic differentiation of BM-MSCs was better than single factor alone.

Conclusion: Our study proved the possibility of isolation of BM- MSCs and in vitro induction into cells expressing most important characteristics of functioning hepatocytes. Our thesis implied the hope of possible therapeutic application of such hepatic lineage against liver diseases in the future. Stem cell research is being pursued vigorously in our laboratories in the hope of achieving major medical breakthroughs in the future.

Keywords: Adult Stem Cells, Mesenchymal Stem Cells, Hepatocytes, Flow Cytometer, RT-PCR

Ps-81: Preparation of Testicular Cell Conditioned Medium from Rat Testis

Mortazavi M, Mohammadi Roushandeh A*

Molecular Genetic Research Center, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
Corresponding Email: a.mohammadiroshandeh@umsha.ac.ir

Objective: In vivo, growth factors that mediate local cell-cell interactions are responsible for the differentiation of primordial germ cells (PGCs) into germ stem cells after the arrival at the genital ridge. in vitro differentiation of PGCs is also dependent on their sequential exposure and response to an array of growth factors. In the presence of multiple growth signals, PGCs restart rapid proliferation in vitro and transform into pluripotent embryonic germ cells (EGCs). Hence, some cases of sterility are due to lack of germ cells, in the future, this problem could be treated using stem cell therapy. Thus, in this study we effort the preparation of testicular cell conditioned medium from rat testis.

Materials and Methods: Testes of 1-day-old newborn
male rats were removed and placed into trypsin-EDTA solution. Then the harvested cells cultured with DMEM medium supplemented with 10% FCS, 1% nonessential amino acids, and 1% penicillin/streptomycin. After proliferation of germ cells, testicular conditioned medium was collected every 3 days starting 10 days after initiation.

**Results:** An obvious germ cell proliferation was evident in TCCs 8-10 days from initiation. The round cells were clustered together and easily dissociated from other cells when dishes were shaken. Germ cells differed in size and appeared as single cells or attached to each other, forming pairs or rows.

**Conclusion:** Testis, particularly of newborns, contains germ cells that release growth factors during in vivo culture. In this regard, we try the in vivo differentiation of mesenchymal stem cells of rats into PGCs and precursors of sperm by TCC medium induction. Their results will be announced later.

**Keywords:** TCC, Testis, Germ Cell , Rat

**Ps-82:** Evaluating the Expression of miR-491 As A Potential Stemness Marker in Lung Tumor and Non-Tumor Tissue

Mowla SJ, Naeli P, Mirzadeh Azad F, Tavallaee M

1. Molecular Genetics Department, Faculty of biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

**Corresponding Email:** sjmowla@yahoo.com

**Objective:** MicroRNAs (miRNAs) are small non-coding regulatory RNA involved in diverse biological processes, including development, differentiation, proliferation, and apoptosis. It is approved that abnormal expression of miRNAs could cause various diseases, including cancer. P53 is a master negative regulator of Nanog expression which is a key to maintenance of pluripotency in stem cells, thus loss of p53 promotes stemness and self-renewal. miR-491 is known to negatively regulate TP53 therefore its aberrant expression could negatively affect TP53 regulatory pathway. Considering the potential role of miR-491 to miss-regulate p53 and based on cancer stem cell (CSCs) hypothesis, deregulation of miR-491 might ignite the tumorigenic process in adult stem cell and cancer stem cell.

**Materials and Methods:** In this study, RNA was extracted from lung tumor and non-tumor tissues. Real-time reverse transcription polymerase chain reaction (RT-PCR) assays were performed with specific stem-loop primer and EVA green master mix. The ability of primer to amplify specific microRNA was evaluated. Results: According to our data, stem-loop primer could amplify miR-491-5p specifically. RT-PCR results showed that expression level of miR-491-5p could be evaluated in lung tumor and non-tumor tissue. In addition, expression level of miR-491-5p might be significantly different in lung tumor and non-tumor tissue.

**Conclusion:** Our data suggested a potential role of miR-491-5p in tumorigenesis. According our data and previous data we can say that miR-491 might have a role in stemness of stem cell and cancer stem cell.

**Keywords:** MicroRNA, Cancer Stem Cell, miR-491-5p, Lung Cancer

**Ps-83:** Evaluating The Expression Alteration of miR-296 As A Stem Cell Specific Marker in Lung Tumor

Mowla SJ, Mirzadeh Azad F, Tavallaee M, Ghanei M, Soltani B

1. Department of Genetics, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran
2. Human Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

**Corresponding Email:** sjmowla@modares.ac.ir

**Objective:** MicroRNAs (miRNAs) are a group of non-coding regulatory RNA involved in diverse biological processes, including development, differentiation, proliferation, and apoptosis. miRNA genes are commonly found in cancer-associated genomic regions and their expression pattern alterations are reported in several cancers, including lung cancer. miR-296 is a stem cell specific microRNA which is located in 20q13.32 genomic locus. By independent experiment on genomic analysis of cancer cell lines, it is shown that this chromosome region is amplified in lung cancer. Furthermore, miR-296 is up regulated during human cell immortalization and in embryonic stem cells. According to the cancer stem cells (CSCs) hypothesis, aberrant expression of such a gene might contribute to tumorigenicity. Herein, our aim of study is to evaluate the expression alteration of miR-296 as a cancer stem cell specific marker in lung tumor tissues.

**Materials and Methods:** To investigate miR-296 expression pattern in a matched case-control way, formalin-free paraffin-embedded (FFPE) samples of lung tumor along with matched non-tumor margin were obtained. Paraffin was removed using the xylene-ethanol method then prepared tissues were treated by proteinase K to eliminate inhibitory proteins. Total RNA was isolated using TRIzol reagent. Specific cDNA synthesized by means of stem-loop primer and miRNA expression assessed by real-time PCR.

**Results:** Our data revealed that designed stem-loop primer was highly specific and could sucessfully assess the expression alteration pattern of miR-296 in lung tumor versus non-tumor samples.

**Conclusion:** According to our primary set of data, the expression of miR-296 in lung tumor could claim the presence of CSCs in tumor cell population. However, in-
Ps-84: Induced Pluripotent Stem Cells Generated from Adult Bone Marrow Derived Cells of The Non-Human Primate (Callithrix jacchus) Using A Novel Quad-Cistronic and Excisable Lentiviral Vector

Mueller Th*, Wiedemann A¹, Glage S¹, Blasczyk R¹, Bernemann I¹, Hemmer K¹, Figueiredo C¹, Pogozhykh O¹, Schambach A¹, Schwamborn J¹
1. Institute for Transfusion Medicine, Hannover Medical School, Hannover, Germany
2. Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Germany
3. Institute for Transplantation Medicine, Hannover Medical School, Hannover, Germany
4. Institute of Cell Biology, Westfaelian-Wilhelms-University, Muenster, Germany
5. Institute for Experimental Hematology, Hannover Medical School, Hannover, Germany

Corresponding Email: mueller.thomas@mh-hannover.de

Objective: Regenerative medicine is in need of solid, large animal models as a link between rodents and humans to evaluate the functionality, immunogenicity, and clinical safety of stem cell-derived cell types. The common marmoset (Callithrix jaccus) is an excellent large animal model, genetically close to humans and readily used worldwide in clinical research. Until now, only two groups showed the generation of induced pluripotent stem cells (iPSCs) from the common marmoset using integrating retroviral vectors.

Materials and Methods: Therefore, we reprogrammed bone marrow-derived mesenchymal cells (MSCs) of adult marmosets in the presence of TAV, SB431542, PD0325901, and ascorbic acid via a novel, excisable lentiviral spleen focus-forming virus (SFFV)-driven quad-cistronic vector system (OCT3/4, KLF4, SOX2, C-MYC).

Results: Endogenous pluripotency markers like OCT3/4, KLF4, SOX2, C-MYC, LIN28, NANOG, and strong alkaline phosphatase signals were detected. Exogenous genes were silenced and additionally the cassette was removed with a retroviral Gag precursor system. The cell line could be cultured in absence of leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) and could be successfully differentiated into embryoid bodies and teratomas with presence of all three germ layers. Directed differentiation generated neural progenitors, megakaryocytes, adipocytes, chondrocytes, and osteogenic cells.

Conclusion: Thus, all criteria for fully reprogrammed bone marrow-MSCs of a nonhuman primate with a genetically sophisticated construct could be demonstrated. These cells will be a promising tool for future autologous transplantations.

Keywords: Common Marmoset, Induced Pluripotent Stem Cells, Excisable Lentiviral Vector, Bone Marrow Derived MSCs

Ps-85: Computational Fluid Dynamic Model to Optimize Air-Lift Bioreactor Geometry for Stem Cell Expansion

Nabavinia M¹,², Alizadeh R², Khoshfetrat AB¹,²
1. Tissue Engineering and Stem Cell Research Laboratory, Chemical Engineering Faculty, Sahand University of Technology, Tabriz, Iran
2. Chemical Engineering Faculty, Sahand University of Technology, Tabriz, Iran

Corresponding Email: khoshfetrat@sut.ac.ir

Objective: Air-lift bioreactors can play an important role in tissue engineering because of many advantages, such as simplicity of construction, absence of moving parts, easy sterilization and low power consumption. However, optimum design of airlift bioreactors for cultivation of stem cells is still a difficult task, because of complicated relations between mixing and mass transfer, and also high sensitivity of stem cells to shear stress. In this study, computational fluid dynamics (CFD) analysis using related software was used to characterize an internal loop airlift bioreactor for stem cell proliferation. The effect of geometry parameters such as sparger position and configuration, ratio of the downcomer to the riser area (Ad/Ar), and height of liquid above (Ha) and below (Hb) of the draft tube was considered to identify the complex hydrodynamic environment as well as shear stress applied to suspension culture of stem cells. The results demonstrated that sparger position and configuration has maximum effect on shear stress exposure on cell although shear stress can be reduced with increasing Ha in this area. In addition, large Ad/Ar was found to be useful for minimum exposure shear stress on cells while it has negative effect on cell distribution as same as Hb. Therefore, optimum design of air-lift bioreactor can be achieved by CFD analyzing with comprehensive considering of hydrodynamic environment to apply this type of bioreactor in tissue engineering without performing costly time-consuming experiments.

Materials and Methods: Computational fluid dynamic software (ANSYS 14.0) was used to consider geometric parameters of air-lift bio-reactor hydrodynamic environment to apply this kind of bio-reactor in tissue engineering for expanding stem cells.

Results: The effect of geometry parameters such as sparger position and configuration, ratio of the downcomer to the riser area (Ad/Ar), and height of liquid above (Ha) and below (Hb) of the draft tube was considered to identify the complex hydrodynamic environment as well as shear stress applied to suspension culture of stem cells. The results demonstrated that sparger position and configuration has maximum effect on shear stress exposure on cell because of effected on bubble formation and geometry. Although shear stress can be reduced with increasing Ha in this area, in addition, large Ad/Ar was found to be useful for minimum exposure shear stress on cells while it has negative effect on cell distribution as same as Hb.

Conclusion: As the above results show, optimum design...
of air-lift bio-reactor can be achieved by CFD analyzing with comprehensive considering of hydrodynamic environment to evaluate applicability of air-lift bio-reactor in tissue engineering without performing costly time-consuming experiments.

**Keywords:** Air-Lift Bioreactor, Geometry Optimization, Stem Cell, CFD

**Ps-86: Biomimetic Scaffold to Enhance Chondrogenic Differentiation of Mesenchymal Stem Cells**

Naghizadeh Z1*, Karkhaneh A2, Shokrgozar MA3, Bonakdar Sh1, Solouk A2

1. Biomedical Engineering Faculty, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran
3. National Cell Bank of Iran (NCBI), Pasteur Institute of Iran, Tehran, Iran

**Corresponding Email:** ziba.naghizadeh@gmail.com

**Objective:** In this study, the differentiation behavior of Mesenchymal Stem Cells (MSCs) onto the surfaces of blended semi-interpenetrating polymer network(semi-IPN) scaffolds consist of Polycaprolactone (PCL), Polyvinyl alcohol (PVA) and Gelatin (GEL) in order to mimic natural cartilage extracellular matrix were investigated.

**Materials and Methods:** PCL-PVA-GEL blended semi-IPN scaffolds were prepared by mixing aqueous and non-aqueous polymer solutions followed by homogenizing, and freeze-drying. Then, the scaffolds were characterized by SEM, compressive mechanical test, and biological assays of MSCs culture, MTT, DMMB, and AO/PI.

**Results:** Cartilage lesions are usually irreparable and natural physiological response is not effective on their treatment. Therefore, tissue engineering using polymeric scaffolds in order to create the tissue with similar properties to the cartilage is an interesting and promising procedure. In this study, the results showed using PCL-PVA-GEL com- position with aforementioned procedure could lead to an open interconnected porous structure with similar modulus ($1.27 \pm 0.04$ MPa) to the natural human cartilage tissue. The surface of scaffolds showed an excellent efficiency in the adhesion and proliferation of MSCs. The increase of cell proliferation and secretion ECM proteins was achieved through affinity of cells possibility toward the GEL containing matrix. A significant increase in proteoglycan content from $5.4 \pm 1.13 \mu g/ml$ to $9.81 \pm 1.74 \mu g/ml$ was observed after 21 days. The results of AO/PI test revealed more than 90% of cells were alive inside the scaffolds.

**Conclusion:** The study revealed that the aforementioned scaffold as a blend of natural and synthetic polymers may be used as a promising substrate in tissue engineering for cartilage repair using MSCs transplantation.

**Keywords:** PCL, PVA, GEL, Cartilage, MSC Differentiation

**Ps-87: Effects of Hydrostatic Pressure on Chondrocyte Biosynthesis in Polycaprolactone-Polyvinyl Alcohol-Gelatin Scaffolds**

Naghizadeh Z1*, Karkhaneh A2, Shokrgozar MA3, Bonakdar Sh1, Solouk A2, Haghighipour N2

1. Biomedical Engineering Faculty, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran
3. National Cell Bank of Iran (NCBI), Pasteur Institute of Iran, Tehran, Iran

**Corresponding Email:** ziba.naghizadeh@gmail.com

**Objective:** This study was undertaken to determine the effects of hydrostatic pressure on the biochemical properties and gene expression of mesenchymal stem cells (MSCs) onto PCL (polycaprolactone) -PVA (polyvinyl alcohol)-GEL (Gelatin) semi IPN scaffolds in cartilage tissue engineering.

**Materials and Methods:** TheMSC cells were seeded onto PCL-PVA-GEL semi IPN scaffolds in two groups (A and B) for 7 and 21 days, respectively, and then loaded with hydrostatic pressure (5 MPa, 0.5 Hz) for 2 hours per day for the period of 7 days and compared with two non-loaded groups (C and D) as control. DMMB and Real-time PCR analysis for assaying cartilage-specific ECM gene markers were carried out.

**Results:** Mechanical stimuli such as hydrostatic pressure, which plays a considerable role in the mecha-noregulation of cartilage, can be sensed by chondrocytes in articular cartilage. According to the results, although no significant differences for GAG amount between the loaded and non-loaded constructs was observed after 14 days, significant and considerable increases in the expression amount of type II collagen mRNA levels in group A (from $2.43 \times 10^{-4} \pm 5.32 \times 10^{-5}$ to $2.09 \times 10^{-2} \pm 1.07 \times 10^{-4}$ time), and in group B (from $3.04 \times 10^{-4} \pm 4.31 \times 10^{-5}$ to $2.08 \times 10^{-3} \pm 1.59 \times 10^{-4}$ time) in comparison with non-loaded groups (C and D) were observed, respectively. On the other hand, neither significant increase in aggrecan nor in sox9 expression after 14 days was found.

**Conclusion:** Results showed the beneficial role of hydrostatic pressure on the increase of type II collagen mRNA levels in articular cartilage tissue engineering.

**Keywords:** Hydrostatic Pressure, Cartilage, Scaffold, MSC Differentiation

**Ps-88: Endometrial Stem Cells Neuronal Differentiation Ability: A Perspective for Nerve Regeneration?**

Navaei-Nigjeh M

Department of Tissue Engineering, Faculty of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

**Corresponding Email:** mona.navaee@yahoo.com

**Objective:** The human endometrium is a dynamic tissue, which undergoes cycles of growth and regression with each menstrual cycle. Endometrial regeneration also
follows parturition and extensive resection and occurs in postmenopausal women taking estrogen replacement therapy. It was demonstrated that human endometrium contains a low number of endometrial stem cells (EnSCs) which seem to belong to the family of the mesenchymal stem cells (MSCs). Therefore, endometrium may be an alternative source of MSC-like cells for tissue engineering purposes, obtainable with no extra morbidity than that required for other sources of stem cells. The evidence that endometrial-derived stem cells were able to transdifferentiate into specialized cells of tissues different from endometrium, in particular into nervous cells, opened up the possibility of using endometrial-derived stem cells to substitute damaged neurons, that are normally not replaced but lost, in order to repair the Nervous System.

Materials and Methods: The evidence that endometrial-derived stem cells were able to transdifferentiate into specialized cells of tissues different from endometrium, in particular into nervous cells, opened up the possibility of using endometrial-derived stem cells to substitute damaged neurons, that are normally not replaced but lost, in order to repair the Nervous System.

Results: Previous studies have shown EnSCs can respond to signalling molecules that are usually used as standards in neural differentiation and can programme cholinergic and dopaminergic neuronal cells. By inducing cholinergic neurons from EnSCs in a chemically defined medium, we could produce human neural cells required for other sources of stem cells. The evidence that endometrial-derived stem cells were able to transdifferentiate into specialized cells of tissues different from endometrium, in particular into nervous cells, opened up the possibility of using endometrial-derived stem cells to substitute damaged neurons, that are normally not replaced but lost, in order to repair the Nervous System.

Conclusion: In this review, we have examined the recent progress in this field, looking in particular at the applicability of human endometrial stem cells derived neurons usage to enhance nerve regeneration.

Keywords: Endometrial Stem Cells, Nerve Regeneration

Ps-89: Primary Study of The Effect of Methionine Supplementation on Feather Follicles Creation in The Chicken Embryos

Nazem MN1*, Tavakoli H2, Amanollahi R1, Dehghani Tafti E3

1. Department of Basic science, Veterinary faculty, Shahid Bahonar University of Kerman, Kerman, Iran
2. Department of Medical science, Veterinary faculty, Shahid Bahonar University of Kerman, Kerman, Iran
3. Student of faculty of veterinary, Veterinary faculty, Shahid Bahonar University of Kerman, Kerman, Iran
Corresponding Email: nasernazem@yahoo.com

Objective: Methionine is one of the most aminoacids inorder to creation and growth of the hair and feather follicles.

Materials and Methods: 50 fertile eggs from the broiler breeders (Ross 308) devided into 5 equal groups and passed to the hatching machine. Four first groups were inoculated with 20 mg, 30 mg, 40 mg and 50 mg methio-

nie which was dissolved in 0.5 mg sterile water into the yolk sac on the 4th day of incubation. The 5th group was used as the shame control and inoculated with 0.05 ml sterile water into the yolk sac on the 4th day of incubation. On the 18th day of embryonic life, embryous were removed from the eggs and skin samples were taken from the left side of the skin chest and prepared for histological study after hematoxylin and eosin staining.

Results: In the microscopic study, feather follicles were increased in all 4 treatment groups but in the 3rd and 4th groups were significant.

Conclusion: The results of this primary study shows that injection of different doses of methionine can increase the number of follicles during embryonic period significantly (p<0.05).

Keywords: Methionine, Feather Follicles, Chicken Embryos

Ps-90: How RPE Cells Generate Giant Colonies on PolyHEMA and Maintain Their Characteristic Expression of Tyrosinase Gene

Nazemroaya F1*, Soheili ZS1, Deezagi A1, heidari R1, Ahmadi M1, Samiei Sh3

1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Iranian Blood Transfusion Organization Research Center, Tehran, Iran
Corresponding Email: Nazemroaya@yahoo.com

Objective: Retinal pigment epithelial cells (RPE cells) play an important role in the maintenance of normal function of the retina. Tyrosinase is a marker of RPE cells that functions in synthesis of melanin. PolyHEMA refer to poly 2 hydroxy ethyl metacyrlat. Poly 2 hydroxy ethyl matacrylat (PolyHEMA) is a hydrophobic polymer that does not normally support attachment of mamma- lian cells. It is the basic component of contact lenses In the current study we investigated effect of PolyHEMA as a cell culture substrate on viable RPE cells and expression of Tyrosinase gene.

Materials and Methods: A stock solution of poly-HEMA (120 mg/ml) was prepared in 95% ethanol and allowed to be dried under laminar hood then was sterilized by UV and washed by PBS. Human globs were obtained earlier than 24 hours after death from the central eye bank of Islamic Republic of Iran. Neonatal human globs were dissected and RPE cells were isolate and cultured in DMEM: F12 (1:1) supplemented with 10% FBS. Cells between passages 2 and 5 were cultured on PolyHEMA and polystyrene substrates (as control) in 24 wells culture palates. DMEM/F12 was supplemented with 10%FBS or 30%AF or DMEM/F12 serum free me dia were used to nourish cultured cells on polystyrene or PolyHEMA coated vessels. 2 days later MTT assay (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was applied to assess the number of cells RNA
Ps-91: Evaluation of Cytokines Produced by Mitogen Stimulated Bovine Peripheral Blood Mononuclear Cells (PBMCs)

Norian R*, Delirezh N, Morvari A, Beikzadeh B

Department of Microbiology, Veterinary Medicine, Urmia University, Urmia, Iran
Corresponding Email: norian.reza@yahoo.com

Objective: This study was conducted to evaluate cytokine production by bovine peripheral blood mononuclear cells stimulated with phytohemagglutinin (PHA), pokeweed mitogen (PWM) and concanavalin A (ConA). The production of IL-2, IL-4, IL-5, IL-6, IL-10, IL-17 and gamma interferon was measured in the supernatant of stimulated cells. The 2×10^5 cells were cultured in the presence of various concentration of mitogens and cytokine production was assessed using commercially available sandwich ELISA kits.

Materials and Methods: According to the results presented in Table 1 and figure-3.1, IL-2 IL-4, IL-5, IL-10, IL-17 and IFN-γ production in response to PWM mitogens was highest and Con A lowest amount and the median values of three mitogens were in the following order: PWM > PHA > ConA > cell control. In the case of IL-6, the production of this cytokine was the same amount for PWM and Con A and lower amount for PHA stimulation.

Results: The results of this study not only showed a normal range for the production of these cytokines from PBMCs that were affected by mitogens, but it demonstrated that the bovine immune system at 2.5-3 months is postnatally mature enough to mount an effective immune response to mitogens as well as specific antigens

Conclusion: The results demonstrated that the bovine immune system at 2.5-3 months is postnatally mature enough to mount an effective immune response to mitogens as well as specific antigens

Keywords: PHA, Con A, PWM, cytokine, Bovine, PBMCs

Ps-92: The Influence of Cerebrospinal Fluid on Epidermal Neural Crest Stem Cells May Pave the Path for Cell-Based Therapy

Pandamooz S1,2*, Naji M3, Alinezhad F1,4, Zarghami A1,4, Pourghasem M1

1. Cellular and Molecular Biology Research Center, School of Medicine, Babol University of Medical Sciences, Babol, Iran
2. Department of Biology, Faculty of Science, Kharazmi University, Tehran, Iran
3. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
4. Student Research Committee, School of Medicine, Babol University of Medical Sciences, Babol, Iran
Corresponding Email: m.pourghasem@hotmail.com

Objective: A wide range of therapeutic approaches have been evaluated for treatment of central nervous system (CNS) injuries and neurodegenerative disease and epidermal neural crest stem cell (EPI-NCCS) as an adult-resident multipotent stem cell in the bulge of hair follicle has shown a number of advantages which makes it an appropriate cell type for transplantation. A prominent factor in cell based therapy is the practicalities of different routes of administration. Cerebrospinal fluid owing to its adaptive library of secreted growth factors can provide a trophic environment for transplanted cells.

Materials and Methods: In this study, the highly pure population of EPI-NCCS was obtained by virtue of its migratory ability from bulge of hair follicles. Cells were characterized by RT-PCR and immunocytochemistry. Subsequently isolated stem cells were cultured in CSF collected from cisterna magna of adult rat. Isolated cells were treated with CSF for 72 hours and then the expression of pertinent markers was assessed at the gene and protein level by real-time PCR and immunocytochemistry. Also Colorimetric immunoassay quantified the rate of proliferation.

Results: Our results revealed that isolated EPI-NCCS could survive in the CSF and they proceeded expression of Nestin, β - Tubulin III (early neuronal marker) and GFAP (glia marker) in this environment. Also colorimetric BrdU ELISA kit indicated CSF decreased significantly the proliferation rate of EPI-NCCS compared to expansion culture medium (p<0.01).

Conclusion: Alltogether, CSF as a cocktail of growth factors helps EPI-NCCS to acquire some desirable traits and due to its circulatory system that is in close contact with different parts of CNS, can be a practical route of administration for delivery of injected stem cells into injured spinal cord. Moreover, low proliferation rate of these stem cells in CSF is an attractive feature in cell-based therapy.

Keywords: Epidermal Neural Crest Stem Cell, Cerebrospinal Fluid, Hair Follicle, Bulge

Ps-93: Study of Effect of Mouse Embryos Lower Limb-Buds Co-Culture System on Differentiation of Mesenchymal Stem Cell to Neurons

Porlarimi FZ1*, Khan Babaei R2, Naji T3, Porlarimi H4

1. Department of Cell and Molecular Biology, Pharmaceutical
Kord M2, Ebrahimi H2, Pourrajab F1, 2 *, Hekmati Moghaddam SH 1, 3 , Forouzannia SK 1, 4 , Babaeizarch M 1, Tajik Kord M 1, Hekmatimoghaddam SH 1, 3 , Forouzannia SK 1, 4 , Babaeizarch M 1, Tajik Kord M 1

Corresponding Email: fatemeh.zahra_porlarimi@yahoo.com

Objective: One kind of adult stem cell that are most important and attracted the attention of most researchers is mesenchymal stem cell. Mesenchymal stem cell can differentiate in vitro into various kinds of mesenchymal cells such as cartilage, fat, muscle and non-mesenchymal cells including neural cells. In this study lower limb-bud tails and feet of mouse embryos co-culture have been used for differentiation of mesenchymal stem cell into nerve cells.

Materials and Methods: Mice Balb/C were killed, then bone marrow of femur bone were removed and cultured in culture flask containing DMEM/f12 subelement with %15fBS and penicillin-streptomycin antibiotic. The medium was changed once every 48 hours, after confluency, have been passage. After the third and fourth passage, mesenchymal stem cells co-cultured with lower limb-bud(tail and feet) respectively. One week later, the morphology of cells evaluated by kerziel viole staining for confirming the differentiation of Msc to neurons. We used flowcytometry for assessment of specific marker neural stem cells.

Results: The result of this study showed that after two weeks of primary culture, Fusi Form like-fibroblast cells was observed with 70-75 percent confluency. Staining showed, morphologically modifications observed after differentiation were similar to morphologically modifications that occurs in neurons cells. Flowcytometry analysis showed that cells after induction will express neuronal marker Nestin and β-tubulin.

Conclusion: In this study of lower limb buds co-culture system, tail and feet for differentiation of mesenchymal stem cells into neuron cell are used. Result showed that feeder layer cells within secretion of growth factor, FGF, EGF, RA cause is differentian of mesenchymal stem cell into neurons. These data confirm that in vitro hMSCs can differentiation to neurons cells.

Keywords: Mesenchymal Stem Cell, Co-Culture, Lower Limb Buds, Differentiation

Ps-94: Combination of Final Blood Products Improves The Efficacy of Autologous Bone Marrow Stem Cell Therapy

Pourrajab F1, 2*, Hekmati Moghaddam SH1, 3, Forouzannia SK1, Baqhiyazdi M1, Babaeizarch M1, Tajik Kord M3, Ebrahimimi H3

1. Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Objective: Isolation procedures of autologous BMSCs might affect cell functionality and therapeutic efficacy. The aim of this study was to identify an association between combination of blood products and the quality and functional activity of bone marrow derived progenitor cells (BMSCs) processed by special protocol and used for cardiovascular regenerative therapies.

Materials and Methods: Quality of cell isolation was assessed by measuring the total number of isolated BMSCs, CD19+, CD34+ and CD45+ cells, their colony-forming unit (CFU) and invasion capacity, cell viability, and contamination of the final BMC preparation with thrombocytes and red blood cells (RBCs).

Results: The number of final BMSC and the cell proliferation capacity significantly correlated with reduced isolation procedures, and a combination of autologous BMSCs with final blood products. Higher numbers of BMSC in the cell preparation were associated with increased BMSC viability, CFU capacity, and invasion potential.

Conclusion: Combination treatment of autologous BMSCs affects the functionality of expanded BMSCs and determines the extent of their proliferation and their viability. These results suggest a bioactivity response relationship very much like a dose-response relationship in drug trials.

Keywords: Isolation Procedures, Autologous BMSCs, Blood Products, Proliferation

Ps-95: Human Umbilical Cord Wharton’s Jelly Stem Cells (hWJSCs) Inhibit Human Myeloid Leukemia Cell Growth, but Not Amniotic Stem Cells

Pourrajab F 1*, Tajik Kord M 3, Hekmatimoghaddam Sh 2, Zareh F 3, Asadisaghandi A 3, Deganhamshadi M 1

1. Department of Clinical Biochemistry and Molecular Biology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. School of Paramedicine, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Corresponding Email: mina_poorrajab@yahoo.com

Objective: The umbilical cord and placenta are extra-embryonic tissues of particular interest for regenerative medicine. Although traditionally discarded upon birth, the placenta and umbilical cord are now regarded as a valuable source of cells with stem cell-like plasticity. Umbilical cord mesenchymal stem cells (MSCs) have been shown to inhibit breast cancer cell growth but it is...
not known whether this effect is specific to only breast cancer cells.

**Materials and Methods:** We compared the effects of human Wharton’s jelly stem cell (hWJSC) and amniotic stem cell (hASC) co-cultures on human myelogenous leukemia cell line K562. The cells were co-cultured with either hWJSC (confluence 60%) or hASC for 24-72 hours and changes in cell morphology, proliferation, and cell death were studied.

**Results:** All three time course co-cultures of cancer cells with hWJSCs showed growth inhibition with cell death (10-50%), accompanied with cell shrinkage and blebbing, while growth assay of cancer cell co-cultures with hASCs showed no considerable growth inhibition (by 4-9%). Cell cycle assays showed increases in G1 phase for all three time course co-cultures.

**Conclusion:** Suggestive, hWJSCs possess tumor inhibitory properties that are not specific to breast cancer cells alone and these effects are mediated via agents in their growth media.

**Keywords:** Human Wharton’s Jelly Stem Cells, Amniotic Stem Cell, Human Myelogenous Leukemia Cell Line, Tumor Inhibitory Properties

Ps-96: Design of A Combinatorial and Feasible Protocol for Autologous Bone Marrow Stem Cell Transplantation in Patients Candidate for CABG

Pourrajab F1,2*, Hekmatimoghaddam SH1,3, Forouzannia SK1, Baqhiyazdi M1, Babaezarch M1, Ebrahimii H2

1. Yazd Cardiovascular Research Center, Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Clinical Biochemistry and Molecular Biology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. School of Paramedicine, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

**Objective:** Despite advances in revascularization therapy, coronary artery disease (CAD) posing a leading cause of morbidity and mortality risks worldwide, continues to challenge the medical community. This study aimed at examining the efficacy of bone marrow stem cells combination therapy used for cardiovascular regenerative therapies, and also to identify isolation procedure of autologous bone marrow stem cells (ABMSCs) might affect cell functionality and therapeutic efficacy, administered through intra-myocardial and during coronary artery bypass graft (CABG).

**Materials and Methods:** The Yazd Cardiovascular research center was investigating to establish a xeno-free protocol with less processing steps for cell therapy and treatment of cardiovascular disease. After thoughtful consideration of both statistical and clinical principles, we recruited 15 patients to receive either combinations of ABMSCs or placebo. The overall baseline age of the patients was 60 ± 9 years, and due to severe ischemic cardiomyopathy, need to have coronary bypass surgery. Each patient received a median of 5×10^6 ± 1×10^6 ABMSC combinations harvested by the special procedure or an equal volume of diluent (placebo). Changes in left ventricular ejection fraction from baseline and wall motion, examined by magnetic resonance imaging and by echocardiography, respectively.

**Results:** ABMSC delivery was successful in all patients with no arrhythmias, no elevated cardiac enzymes or complications related to the delivery. Compared with the placebo-control group, the ABMSC group had significant changes in routine clinical and biochemical test values (p < 0.05). Weekly angina frequency was significantly lower in the cell-injected group than in placebo group. Left ventricular ejection fraction, end-systolic volume and wall motion was found to be significantly improved in the ABMSC group compared to the placebo group over the follow-up period.

**Conclusion:** The designed protocol should be considered as an alternative method of ABMSC application in clinical trial for improving blood flow and contractile function of the heart, and also to safely deliver large number of cells regardless of different stem cell processing steps and RBC or xeno-contamination. This study indicates that the beneficial effect of special way which we used for ABMSC transplantation, would be pronounced in CAD patients.

**Keywords:** Autologous Bone Marrow Stem Cells, Isolation Procedure, Regenerative Therapies, Coronary Artery Bypass Graft

Ps-97: Study on Lactobacillus of European Cat Fish (Silurus Glanis) Intestine in Siahdarvishan River of Iran by PCR Method

Pouyafar AR1,2*, Mortazavi SJ2, Babazadeh D1, Sadeghi AR1

1. Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Department of Aquatic Disease and Hygiene, Tabriz Branch, Islamic Azad University, Tabriz, Iran

**Objective:** Lactic acid bacteria (LAB) are constituted of a heterogeneous group of Gram-positive bacteria with a strictly fermentative metabolism from which lactic acid is a key metabolite.

**Materials and Methods:** During present research about 50 European cat fish (Silurus glanis) were hunted from siahdarvishan river (Bandar Anzali, Iran) randomly. The samples were transported to the Microbiology laboratory under sterile conditions and in the vicinity of ice. After dissection according to sterile Method; 1 gram of stool was sampled from the anterior part of intestine and cultured in MRS Agar plates. Phonotypical and biochemical of Bacteria was identified for molecular characterization distinction of lactobacillus species. 16SrDNA gene was amplified and after electrophoresing cutting pattern of 16s rRNA, compared by Gen Doc program with cutting
pattern of 16s rRNA gene sequences of species which recorded in the Gene Bank (NCBI).**

**Results:** In biochemical tests, 15 samples were positive for Lactobacillus, 7 cases of them were lactobacillus plantarum and 3 cases were lactobacillus casei. Cutting patterns of 5 other samples were different from L. Casei, L. plantarum, and even from cutting patterns of 16s rRNA genes of lactobacillus that have been recorded in Bioinformatics.

**Conclusion:** Results indicated 30 percent of European Cat Fish samples were positive for Lactobacillus and the most cases were lactobacillus plantarum.

**Keywords:** Cat Fish, Lactobacillus Plantarum, Lactobacillus Casei

**Ps-98:** The Effects of Fndc5 Overexpression on Characteristics of Mouse Embryonic Stem Cells and the Expression of Three Germ Layer Markers and Mitochondrial Genes

**Rabiee F**, **Forouzanfar M**, **Tanhai S**, **Motavali-Bashi M**, **Ghaedi K**, **Nasr Esfahani MH**

1. Department of Biology, School of Sciences, Isfahan University, Isfahan, Iran
2. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
**Corresponding Email:** kamranghaedi@royaninstitute.org

**Objective:** Fndc5 formerly known as peroxisomal protein, is suggested to be a PGC1-alpha-dependent myokine and is secreted as Irisin, responsible for blunting of white fat tissues. In adult mouse, mRNA expression level of Fndc5 is high in heart, skeletal muscle and brain. Our previous studies have revealed a significant increase in Fndc5 mRNA when mouse embryonic stem cells were differentiated into beating bodies and neural precursor cells. As a step closer to clarify the function of Fndc5 on embryonic stem cell differentiation we have overexpressed Fndc5 in embryonic stem cell to analyse the expression of three germ layers marker and mitochondrial genes.

**Materials and Methods:** Stably transformed overexpressing Fndc5 mouse embryonic stem cells (Rh20-Fndc5) after induction by doxycyclin were cultured for three days. Then, 5×10^5 of treated mESCs were suspended in a non-adhesive bacterial dish by the medium without LIF, and small molecules SB and PD (for stemness maintenance) for six days in the absence of Fndc5 overexpression. Media were changed every 2 days. Total RNA was extracted from cultured cells. cDNA synthesis was carried out with cDNA Synthesis Kit. Real-time PCR was performed to estimate the mRNA expression levels of target genes.

**Results:** The results depicted that expression of ectodermal and mesodermal genes such as Brachyury and nestin was higher in those cells with overexpressing Fndc5 compare with control. Furthermore there were not significant difference in the expression levels of mitochondrial markers in overexpressing Fndc5 group and control.

**Conclusion:** Fndc5 may be a master gene, whose product would modulate the expression of ectodermal and mesodermal genes. However it has not any effect on mitochondrial genes.

**Keywords:** Fndc5 Overexpression, Mouse Embryonic Stem Cells, Ectodermal Markers, Mesodermal Markers

**Ps-99:** Antitumor Activity of Noscapine in Combination with Paclitaxel on Human LNCaP Prostate Cancer Cell Line

**Rabzia A**, **Rashidi Z**, **Khazaei M**

Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
**Corresponding Email:** a.rabzia@yahoo.com

**Objective:** Paclitaxel is generally used to induce apoptosis in most types of cancer cells. Noscapine is an opioid antitussive, and has demonstrated potent antitumor activity. The aim of this study was to investigate the anti-proliferative and proapoptotic activity of noscapine and paclitaxel alone and in combination on human LNCaP prostate cancer cell line.

**Materials and Methods:** LNCaP cells were cultured in RPMI 1640 medium, and were treated by various concentrations of paclitaxel (5, 10, 25, 50 and 100 μM) or noscapine (10, 25, 50 and 100 μM) in three different times (24, 48 and 72 hours). Cell viability and IC50 value was determined using MTT assay. In another set of experiments, the cells were treated with 50nM Paclitaxel combination with different concentrations of noscapine for 48 hours and cell viability and percentages of apoptotic cells was assessed by acridine orange (AO)/ethidium bromide (EB). Data was analyzed by one way ANOVA.

**Results:** The MTT assay showed that paclitaxel and noscapine alone significantly decreased the viability of LNCaP cells in a dose and time-dependent manner, and the IC50 values were 50 nM and 50 μM respectively at 48 hours. Combined treatment with noscapine and paclitaxel significantly decreased in cell viability and increased cells apoptosis, compared to single treatment (p<0.05).

**Conclusion:** Noscapine potentiated the anticancer activity of paclitaxel in a synergistic manner against LNCaP cells.

**Keywords:** Prostate Cancer, Noscapine, Paclitaxel, MTT Assay

**Ps-100:** Evaluation of Cardiac Differentiation Potential of Menstrual Blood versus Bone Marrow-Derived Stem Cells

**Rahimi M**, **Mohseni Kouchesfehani H**, **Zarnani AH**

1. Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University (TMU), Tehran, Iran

**Objective:** The potential of menstrual blood derived stem cells (MB-SCs) was evaluated compared to bone marrow-derived stem cells (BMMSCs) in the context of cardiac differentiation. Given the high similarity of the menstrual blood to the bone marrow as the source of stem cells, the potential of menstrual blood-derived stem cells should be compared to that of bone marrow-derived stem cells.

**Materials and Methods:** Cardiac differentiation was evaluated using a cardiac lineage marker and cardiac troponin I (cTnI). Cardiac differentiation was induced using specific growth factors and extracellular matrix (ECM) components. The differentiation process was monitored by analyzing the expression of cardiac markers using real-time PCR and immunofluorescence analysis.

**Results:** The results showed that MB-SCs exhibited a higher expression of cardiac markers compared to BMMSCs. Furthermore, the MB-SCs were able to form cardiac-like structures, indicating their potential for cardiac differentiation.

**Conclusion:** The results suggest that menstrual blood-derived stem cells have a higher potential for cardiac differentiation compared to bone marrow-derived stem cells, making them a promising source for cardiac regeneration.

**Keywords:** Menstrual Blood, Bone Marrow-Derived Stem Cells, Cardiac Differentiation, Cardiac Troponin I
Objective: Recently, identification of menstrual blood-derived stem cells (MenSCs) as a unique source of stem cell with some characteristics as well as ease of access, high proliferative ability and renewability has created enormous promise for cell therapy. In this study, the differentiation ability of MenSCs into cardiomyocytes has been compared with that of bone marrow-derived stem cells (BMSCs).

Materials and Methods: After characterization of MenSCs compared with BMSCs using flow cytometry, their differentiation into cardiomyocyte was investigated in the presence of 5-azacytidine and basic-fibroblast growth factor. The expression of the putative myogenic cells at mRNA and protein levels was determined by immunofluorescent staining and real-time quantitative PCR.

Results: Based on flow cytometric analysis, the isolated MenSCs typically expressed mesenchymal stem cell markers like CD105, CD73, CD44 and CD166 and lack hematopoietic stem cell markers such as CD34, CD45 and CD133 in a similar manner with BMSCs. However, in contrast to BMSCs, MenSCs exhibited marked expression of OCT4 related to embryonic markers. The both differentiated MenSCs and BMSCs expressed cardiomyocyte markers at mRNA/protein level. While, the expression level of some cardiomyocyte markers such as Connexin-43 and troponin T2 (TNNT2) protein were higher in differentiated MenSCs compared with BMSCs, there was no significant difference between mRNA levels of Connexin-43, Alpha actinin ,Tropomyosin1 and TNNT2 of differentiated MenSCs and differentiated BMSCs.

Conclusion: Based on our data, MenSCs are a unique cell population with differentiation ability into cardiomyocyte-like cells. However, the pattern of cardiac markers at mRNA and protein level in differentiated MenSCs is relatively different with that of derived BMSCs.

Keywords: Menstrual Blood, Bone Marrow, Stem Cell, Cardiomyocyte, Differentiation

Ps-101: Differential Expression of OCT4 Variants in Melanoma Stem Cells

Rajabi Fomeshi M1,2*, Ebrahimim M2, Mowla SJ3

1. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Molecular Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Objective: OCT4, as a transcription factor has a role in stemness maintenance and assumed to be a self-renewal regulator in different kind stem cells including cancer stem cells. It has two spliced variants, OCT4A and OCT4B. Oct4A regulate self-renewal and pluripotency in stem cells, while the function of Oct4B is unknown. The aim of this study is assessment of OCT4A and OCT4B expression in melanoma stem cells.

Materials and Methods: D10 melanoma cell line was grown in complete RPMI medium. The cells were sorted due to the expression of CD133 cell surface marker-one of the most important cancer stem cell (CSC) marker in solid tumors- named CD133+ and CD133- population. Afterward, the expression of OCT4A and OCT4B were assessed in unsorted, CD133+ and CD133- population with specific primers. The paired two-tailed Student's t test was used to compare groups and P value less than 0.05 was considered statistically significant.

Results: Our results revealed that OCT4B variant wasn’t differentially expressed between populations. While OCT4A expression in CD133+ (cancer stem cells) population was significantly more than unfractioned and CD133- population (1.8 fold).

Conclusion: According to this result OCT4A can a candidate for cancer stem cell population and targeting of this factor in cancer stem cells maybe inhibited self-renewal and pluripotency.

Keywords: OCT4A, OCT4B, Melanoma, CD133, Cancer Stem Cell
tion of mesenchymal stem cells, alizarin red staining was used to detect osteoblastic cells.**

**Results:** At the first, MSCs were spindle shape. After affect of extraction during 21 days, osteoblast cells and calcium crystals were observed.

**Conclusion:** The results of the alizarin red staining shows the osteoblastic differentiation and also osteoblastic differentiation of mesenchymal stem cell confirmed that morphogens and osteogenic factors present in cartilage tissue extraction of 13 days old mouse embryo.

**Keywords:** Mesenchymal Stem Cells, Cartilage Tissue Extraction, Osteoblast, 13 Days-Old Mouse Embryo, Cell Differentiation

**Ps-103: Introduction of Scleroderma-Specific Induced Pluripotent Stem Cells in a Dish: An Unlimited and Available Source for Evaluation of Developmental Defects in Regenerative Medicine**

Ranjbar Niavol F1*, Baharvand H1, Aghdami N2

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Regenerative Biomedicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

**Corresponding Email:** nasser.aghdami@royaninstitute.org

**Objective:** Scleroderma—one of the important autoimmune diseases—leads to death of endothelial cells as one of the early events of this disease. The lack of repair after the loss of endothelial cells is observed in these patients and although it is a worldwide disease, its cause has remained unknown. Because of physiological differences between human and other species, a proper scleroderma animal model which represent all aspects of the disease has not been generated yet. So, making a patient-based system to mimic a developmental defect and evaluating its probable repair mechanism is considered as a necessity. In this study it was tried to generate patients’ specific induced pluripotent stem (iPS) cells and characterize them.

**Materials and Methods:** Patient’s fibroblast cells— as an available and unlimited source of disease study in vitro—were isolated, cultured and transduction with retroviral vector containing Oct 3/4, Sox2, Klf4 and c-Myc. For characterization of generated iPS cells, their expression of endogenous pluripotent and silencing of retroviral vector containing Oct 3/4, Sox2, Klf4 and c-Myc. For characterization of generated iPS cells, their expression of endogenous pluripotent and silencing of retroviral genes were evaluated by RT-PCR technique and the existence of pluripotency proteins were assessed by immunostaining. Spontaneous differentiation into endothelial cells was done by embroy body formation and the expression of specific endothelial cell markers were examined by flowcytometry technique.

**Results:** RT-PCR analysis and immunostaining study of generated cells compared to human embryonic stem cells (royan H5) results showed that reprogrammed cells express specific markers similar to embryonic stem cells. EB formation results And also, spontaneous differentiation into endothelial cells using EB formation showed that our scleroderma derived pluripotent cells have differentiation potential like embryonic stem cells.

**Conclusion:** Our study showed that scleroderma specific IPS cells can be indicated as an unlimited and available source for assessing molecular defects in vitro and finding regenerative methods to repair endothelial cells in scleroderma patients.

**Keywords:** Reprogramming, Scleroderma Patient-Specific Induced Pluripotent Stem Cell, Endogenous and Exogenous Gene, Embryoid Body, Regenerative Medicine, Endothelial Differentiation

**Ps-104: Expression Profile of CDH1 in Human Endometrial Tissue During the Menstrual Cycle**

Rastegari E1, 2*, Mahdian S2, 3, Afkarian P2, Shahhoseini M2

1. Department of Cellular and Molecular Biology, University of Science and Culture, Tehran, Iran
2. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

**Corresponding Email:** m.shahhoseini@royaninstitute.org

**Objective:** Endometrium is a specialized tissue that undergoes sequential phases of proliferative and secretory changes during the menstrual cycle, in order to support the implantation and growth of an embryo that are controlled by ovarian steroid hormones. Both estrogen and progesterone, through their receptors, stimulate the expression of key molecules in menstrual cycle, such as genes involved in cell adhesion events. Cadherins are major cell-cell adhesion molecules. E-cadherin is a transmembrane glycoprotein and exists as a cell-to-cell homophilic adhesion molecule at the adherens junction. In this study, the expression of E-cadherin coding gene (CDH1) has been evaluated in human endometrial tissue during menstrual cycle.

**Materials and Methods:** Local ethical approval was gained for this study and informed consent was given by patients. All the women taking part in the investigation had regular cycles, showed no evidence of any pathological uterine disorder and had not used oral contraception or an intrauterine device in the previous 3 months. Cellular RNA was extracted from tissue samples and cDNA synthesis was performed on them. The expression level of CDH1 was evauated by the use of qRT-PCR technique.

**Results:** There were some variations in mRNA expression of CDH1 gene in normal endometrium during menstrual cycle.

**Conclusion:** This finding suggests the dynamic role of CDH1 in endometrium reconstruction during the menstrual cycle.

**Keywords:** Endometrium, E-Cadherin, CDH1, Menstrual Cycle
Ps-105: HGF mRNA Expression in Mesenchymal Stem Cell Like Cells from Glioma Tumor Tissue Samples Treated with Genistein
Razmkhah M1, Chenari N1, Sharifirad M2, Ghaderi A1, Taghipour M2
1. Department of Immunology, Shiraz Institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Neurosurgery, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
Corresponding Email: mrazmkhah@yahoo.com

Objective: Despite advances in surgical and adjuvant radiation therapy and chemotherapy strategies, malignant gliomas continue to be associated with poor prognosis in patients. To address suitable strategies, scientists pay close attention to factors that cause angiogenesis. Angiogenesis is a possible target in the treatment of human gliomas. Genistein has been previously introduced as an anti-angiogenic agent for different cancers. To evaluate the effect of Genistein on the angiogenic cascade, we investigated the expression of hepatocyte growth factor (HGF) transcripts in mesenchymal stem cell like cells from high grade and low grade brain tumors before and after treatment with Genistein.

Materials and Methods: Brain tumor obtained from patient diagnoses with GBM and low grade glioma in sterile condition, wash with PBS, cut in to small pieces, digested with collagenase type I and culture in tissue culture flask contain MDEM 10% FBS. As cells arise to passage 3, they were treated with Genistein and then the HGF gene expression was determined by QRT-PCR in treated and untreated cells.

Results: Genistein has a significant effect on both types of tumors after cells expose to compound compared to untreated cells. According to the results, Genistein has a remarkable effect on downregulating HGF transcripts where it showed 13×106- and 2-fold lower expression than untreated cells. According to the results, Genistein has a remarkable effect on downregulating HGF transcripts where it showed 13×106- and 2-fold lower expression than untreated cells.

Conclusion: Our research showed that Genistein has a significant effect on downregulation of HGF. Consequently, Genistein have some important advantages for treatment of invasive brain tumors, however more investment cannot be ignored to make a suitable decision about effect of this compound on brain tumors.

Keywords: Glioma Tumor, Angiogenesis, HGF, Genistein

Ps-106: Effect of Adipose Derived Stem Cells (ASCs) on EMT Specific Markers after Transfection of MDA-MB-468 Cell Line by An Anti-Angiogenic Chemokine
Razmkhah M1, Haghighifard M1,2, Hosseini A1, Chenari N1, Ghaderi A1,2
1. Shiraz Institute for Cancer Research, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

Objective: Epithelial-mesenchymal transition (EMT) is an important mechanism in tumor progression and metastasis. In addition to the uncontrolled epithelial proliferation and angiogenesis, the role of this mechanism in solid tumors such as breast cancer is also important for cancer progression and metastasis. The role of cytokines such as TGF-β and SDF-1 in triggering the EMT mechanism is previously demonstrated. In contrast to the SDF-1 with angiogenic roles, the chemokine IP-10 has been documented with anti-angiogenic activities but there is no research which shows the effect of IP-10 on EMT. Thus, in this study we investigated the effect of IP-10 overexpression on EMT mechanism in tumor cells. Besides, because of the key role of tumor microenvironment on tumor progression and the importance of ASCs, we also investigated the effect of these cells on the EMT mechanism.

Materials and Methods: ASCs were isolated from 6 breast cancer patients and 6 normal individuals and then cultured. MDA-MB-468 breast cancer cell line was transfected by IP-10 plasmid through electroporation. Transfected cell line was co-cultured with ASCs using transwell system. After 4 days, proteins were extracted and the expressions of MMP9 and MMP13 were defined by western blotting.

Results: Results of western blot for MMP9 and MMP13 protein expressions showed that the expression of both proteins were downregulated in IP-10 transfected MDA-MB-468 cells compared to the untransfected cells. Presence of ASCs causes the upregulation of MMP9 in MDA-MB468 cell line.

Conclusion: Based on the results of this study, IP-10 may downregulate the EMT markers in MDA-MB-468 cancer cell line. No different was found between the ASCs from different sources but the presence of ASCs in tumor microenvironment can lead to the up-regulation of EMT markers. Accordingly, IP-10 may use as an anti-tumor therapeutic agent to suppress tumor progression and metastasis.

Keywords: Adipose Derived Stem Cells, EMT, Breast Cancer

Ps-107: Assessment The Viability of Mesenchymal Stem Cell Like Cells from Meningioam, Low Grade and High Grade Brain Tumor Samples after Treatment with Genistein
Razmkhah M1*, Taghipour M2, Sharifirad M2, Chenari N1, Ghaderi A1,3
1. Shiraz Institute for Cancer Research, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Neurosurgery, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Immunology, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
Corresponding Email: mrazmkhah@yahoo.com

Objective: Despite advances in surgical and adjuvant radiation therapy and chemotherapy strategies, malignant gliomas continue to be associated with poor prognosis in patients. To address suitable strategies, scientists pay close attention to factors that cause angiogenesis. Angiogenesis is a possible target in the treatment of human gliomas. Genistein has been previously introduced as an anti-angiogenic agent for different cancers. To evaluate the effect of Genistein on the angiogenic cascade, we investigated the expression of hepatocyte growth factor (HGF) transcripts in mesenchymal stem cell like cells from high grade and low grade brain tumors before and after treatment with Genistein.
**Objective:** Brain tumors are mostly developing tumors with low survival rate worldwide. Among different types of brain tumors, high grade gliomas are not curable while low grade gliomas are well differentiated tumors with better prognosis. Meningiomas are various sets of brain tumors arising from the meninges. Genistein has been recently introduced with anti-proliferative and anti-angiogenic potent effects in a variety of tumors. In this study the effects of Genistein on the proliferation rate and viability of meningioma, low grade and high grade glioma tumor cells was assessed.

**Materials and Methods:** Brain tumor tissues obtained from patients diagnosed with different types of brain tumors were washed with PBS, cut into small pieces, digested with Collagenase type 1 and cultured in the tissue culture flask containing DMEM and 10% FBS. In passage 3 of culture, cells were treated with different concentrations of Genistein (0, 0.01, 1/250, 1/500 and 0.001 M) for 72 hours and then IC 50 was evaluated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Expression of Fas was determined using quantitative Real-Time PCR before and after treatment with Genistein.

**Results:** Tumor cells have plastic adherent ability with mesenchymal like appearance and the ability to be cultured to 10 passages. High grade and low grade gliomas showed approximately 2-fold decrease in viability after treatment with Genistein (p value<0.05). Meningioma cells also showed 1.4-fold vitality decrease post treatment. Expression of Fas transcripts showed statistically significant higher expression in high grade glioma and meningioma samples after Genistein treatment compared to untreated cells (p value<0.05).

**Conclusion:** Results of this study have important implications for the application of Genistein in different types of brain tumors as a therapeutic intervention in future.

**Keywords:** Brain Tumor, Mesenchymal Stem Cell Like Cells, Genistein

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**Ps-108: IDO1, IDO2 , MICA and MICB mRNA Expression in Isolated Adipose Derived Stem Cells (ASCs) from Breast Cancer Patients Compared to Normal Breast Tissues**

Razmkhah M1, Ghaderi A1,2, Bahrami B1,2 *  
1. Institute for Cancer Research, ICR, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran  
2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran  
*Corresponding Email: bahrami_bh@yahoo.com*

**Objective:** Adipose derived stem cells (ASCs) are detected in the tumor microenvironment and have immunomodulatory effects on both innate and adaptive immune responses. ASC-mediated immunosuppression is mainly induced through the secretion of soluble molecules such as IDO1, IDO2, MICA, MICB, NO, PGE2 and HLA-G5. To evaluate the mRNA expression of IDO1, IDO2, MICA & MICB in adipose derived stem cells isolated from breast cancer patients and compare them to those of normal breast tissues.

**Materials and Methods:** ASCs were isolated from 20 breast cancer patients and 10 normal breast adipose tissues obtained by cosmetic mamoplasty using collagenase digestion. The specified genes were assessed by q-RT-PCR method.

**Results:** IDO1 gene expression in ASC cells from breast cancer patients was 2.3 fold more than that of ASCs from normal breasts. mRNA expression of the other genes including IDO2, MICA and MICB, was respectively 2.9 fold, 1.2 fold and 1.9 fold more than those of ASCs from normal breasts. However there was no significant difference between the expression of the indicated genes in ASCs of breast cancer patients and those of normal individuals.

**Conclusion:** In the current study, the mRNA expression of IDO1, IDO2, MICA and MICB had no significant difference between breast cancer and normal breast tissue. This could be because of the relatively small number of samples in each group; therefore further studies on larger number of samples are needed to shed more light on this issue.

**Keywords:** Adipose Derived Stem Cell (ASC), IDO1, IDO2, MICA, MICB, Breast Cancer

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**Ps-109: A Possible Function for mRNA of PPARγ in Human Embryonic Stem Cell-Derived Neural Differentiation upon Retinoic Acid Treatment**

Salamian A1*, Ghaedi K1,2, Nasr Esfahani MH1, Baharvand H3,4  
1. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran  
2. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran  
3. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran  
4. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran  
*Corresponding Email: ahmadsalamian@royaninstitute.org*

**Objective:** PPARγ, a ligand dependent nuclear receptor, play its role as a transcription factor in many of cells especially adipose tissue. In our recent previous studies, although we have shown a significant increase in mRNA level of PPARγ in neural differentiation from human embryonic stem cells (hESCs), a similar pattern in relevant protein level was not detected. To seek additional evidence for how the expression of PPARγ is regulated, we evaluated here two main regulatory systems, proteasome and miRNAs.

**Materials and Methods:** Treated hESCs with noggin and bFGF were induced to differentiate into neuroectodermal cells with retinoic acid for 6 days. Subsequently, cells were collected in TRIzol reagent to extract total RNA for miRNA specific cDNA synthesis, and evaluate miRNA expression. Furthermore, we used MG132 as a proteasome inhibitor to address the question whether
this protein complex can involve in regulation of PPARγ expression. To this end, we treated neuroectodermal cells with MG132 in day 6 RA treatment where we had observed the most expression of PPARγ.

Results: In the case of miRNA, we measured the expression of three miRNAs, miR20, 27, 133, which had been previously reported as miRNAs specifically targeting PPARγ mRNA. We did not observe significant enhancement related to none of the mentioned miRNAs similar to what we detected regarding PPARγ mRNA. Moreover, we investigated involvement of proteasome in this pathway. Although we inhibited proteasome by MG132, we did not increase in PPARγ protein compared to untreated group indicating that proteasome could not involve in protein degradation.

Conclusion: According to our result, it can be concluded that miRNAs and proteasome don’t involve in regulation of PPARγ expression. Possibly, mRNA of PPARγ plays a prominent role in retinoic acid-induced neural differentiation similar to Tpt1 mRNA in mouse embryonic stem cells where there is negative correlation between mRNA and protein level of Tpt1.

Keywords: Human Embryonic Stem Cell, Neural Differentiation, PPARγ, mRNA, Protein

Ps-110: Isolation and Culture of Neonatal Rat’s Oligodendrocyte Precursor Cells

Salehi S*, Valigeigi M, Behzad Behbahani A
Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
Corresponding Email: abbasdashehyan@gmail.com

Objective: Oligodendrocyte is a type of neuroglia cells that plays an important role in transmission of nerve signaling over the axon to the central nervous system in vertebrates. This action is employed by production and wrapping myelin sheath around the axon. Demyelination which is a result of myelin sheath’s destruction following elimination or reduction supportive role of oligodendrocyte has been reported in diseases such as multiple sclerosis or progressive multi focal leukoencephalopathy (PML). Nowadays, with improvement of knowledge in use of stem cells for the treatment of different diseases, many research projects have been designed in order to the isolate, culture and differentiate oligodendrocyte precursor cells (OPCs) in vitro. The purpose of this study is isolating of OPCs and transferring them into the brain and spinal cord of newborn rat with a genetic defect in myelin production. We are expecting the new OPCs migrate and differentiate to oligodendrocyte cells in the rats.

Materials and Methods: To set up the project, the brain cortex tissue of newborn rat would be used. After standard procedures, homogenous cell population of OPCs would prepare in a defined culture medium supplemented with factors such as bFGF and pDGF. To differentiate OPCs from the other cell population in culture medium, Immunohistochmeical assays and some specific OPCs markers would also be investigated. Following in vitro isolation and differentiation of the OPCs we would be able to study various biological and immunological mechanisms diseases related to the OPCs.

Results: If OPCs can be cultured and maintain in vitro, then we would be able to study, different biochemical, physiological and immunological mechanisms affecting these type of neural cells. On the other hand, the ability of oligodendrocytes in myelin production in vivo would also be investigated.

Conclusion: The results would show if we would be able to use OPCs for myelin substitution in patients with myelin sheath’s destruction.

Keywords: Oligodendrocytes, Rat, Multiple Sclerosis

Ps-111: Cartilage-Specific Gene Expression in Equine Mesenchymal Stem Cells in Response to Supplemented Medium with Growth Factors

Shademan M1, Abavisani A1, 2, Dehghani H1, 2, Zahedi M1

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Embryonic and Stem Cell Biology and Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
Corresponding Email: milad.shademan@gmail.com

Objective: Research on the use of mesenchymal stem cells (MSCs) in tissue regeneration has generally been driven by the needs of human and veterinary medicine. Adipose-derived mesenchymal stem cells (AMSCs) have potential to differentiate into various lineages such as cartilage. As first step, the capability of chondrogenic differentiation should be confirmed in vitro.

Materials and Methods: To isolate AMSCs, fat samples were collected from three mares aged 3, 6 and 9- years old. Cells were isolated by mechanical and enzymatic (Collagenase I) process and were cultured in optimized conditions. After 3 subcultures, to analyze chondrogenic differentiation, 500,000 cells were cultured under five different treatments and were differentiated with appropriate chondrogenic medium supplemented with or without TGF-beta3 and BMP-6 in 3D micropellet system for 21 days. Chondrogenic differentiation was assessed by staining with toluidine blue and aggrecan expression as a cartilage-specific gene.

Results: Isolated AMSCs were plastic-adherent, fibroblast-like morphology and expressed specific markers. After the period of chondrogenic culture, histological results confirmed chondrogenic differentiation in both groups containing chondrogenic media with or without growth factors compared with control group. More lacuna formation and more adhesive matrixes was seen in growth factors supplemented groups. Moreover, the band of aggrecan fragment in PCR gels were sharp and most highlighted in group supplemented with two growth factors compared with other group.

Conclusion: The results suggest that addition of growth...
Ps-112: The Pluripotency Properties of Human Placenta-Derived Mesenchymal Stem Cells

Shaer A1, 2*, Azarpira N2, Aghdae A2, Babaee M2

1. Department of Biology, Science and Research Branch, Islamic Azad University, Shiraz, Iran
2. Transplant Research Center, Transplant Research Center, Shiraz University of Medical Science, Shiraz, Iran

Corresponding Email: anahita.shaer@yahoo.com

Objective: The placenta is a complex, sophisticated organ with several important functions throughout gestation. To explore the feasibility of placenta tissue as a reliable and efficient source for generating mesenchymal stem cells (MSC), we studied some of pluripotency properties in these cells.

Materials and Methods: MSC were generated from human placenta tissue by explant. The cells at third passage were characterized for expression of cell surface markers. Placenta derived MSCs were stained with a panel of monoclonal antibodies: CD90, CD44, CD105, CD34 and CD133. Stained cells were re-suspended in PBS, analyzed using FACS Calibur flow cytometer.

Results: Flow cytometry analyses showed that MSCs were a homogeneous cell population, which more than 90% of the cells were positive for mesenchymal markers (CD90, CD44, CD105), and negative for CD34 and CD133.

Conclusion: This study showed that human MSCs were positive for mesenchymal markers, this data indicating their closeness with embryonic stem cells and they are a good novel source for stem cell therapy.

Keywords: Mesenchymal Stem Cell, Placenta, Surface Marker

Ps-113: Production of Recombinant hGDNF in HEK293t Cell Line

Shahbazi S1*, Ghaedi K1, 2, Dormiani K2, Khazaie Y1, Lachinani L1, Foruzanfar M2, Nasr Esfahani MH1

1. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
2. Department of Molecular Biotechnology at Cell Science Research Center; Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Corresponding Email: mh.nasr-esfahani@royaninstitute.org

Objective: Human glial cell line-derived neurotrophic factor (hGDNF) is a disulfide linked homodimeric glycoprotein. This protein is able to protect the dopaminergic neurons and it can also induce regeneration of injured neurons in the central nervous system in vivo. So, it is a very promising protein for the treatment of Parkinson’s disease and other neurodegenerative disorders. GDNF has further critical roles outside the nervous system in the regulation of kidney morphogenesis and spermatogenesis. GDNF needs a multicomponent receptor system, composed of RET and GFRα1 receptors. GDNF family ligands also signal through the neural cell adhesion molecule NCAM. The present work describes a method to obtain high amount of purified hGDNF using a mammalian cell line.

Materials and Methods: The coding sequence of the hGDNF which was fused with the signal sequence at N-terminal and histidine tag at the C-terminal was cloned in the expression vector pBudCE4.1/attB. Then HEK293T cell line was stably transfected with the construction with Lipofectamine. The secreted protein was purified by chromatography and protein expression was analyzed by SDS-PAGE and by Western Blot. The biological activity of the purified GDNF was assessed using the PC12 cell line. For this The expression of the two GDNF receptors, RET and GFRα1, on PC12 cells was confirmed by RT PCR. Then we assayed the ability of purified GDNF to induce neurite outgrowth from this cells.

Results: In this study, the expression vector was constructed and it was transfected successfully into HEK293T cell line and the recombinant biologically active GDNF was obtained.

Conclusion: In this study, we describes a method to obtain high amount of purified hGDNF using a mammalian cell line.

Keywords: hGDNF, HEK293T, PC12, Parkinson’s Disease

Ps-114: Direct Conversion of Human Fibroblasts into Functional Neural Cells Using Reprogramming Strategies

Shahbazi E1*, Nemati Sh1, Kiani S1, Baharvand H1, 2

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, Tehran, Iran

Corresponding Email: baharvand@royaninstitute.org

Objective: Cell fate conversion is not a dream anymore. Not only it is biologically feasible today, but also it is adopted as a new tool in cell biology research within a short time. For several years it was believed that the fate of each individual cell which is established during development is irreversible and irrecoverable due to illness and injury. Successful reprogramming of somatic cells to a pluripotency state using only four transcription factors raises the question whether it is possible to convert a fully differentiated cell type into another directly in vitro, without any additional step like going back to pluripotent state. Our purpose in this project is develop a directed transdifferentiation protocol, for generating multipotent, neural lineage restricted, and nonmutogenic iNSCs from human fibroblasts by direct reprogramming strategy.
Materials and Methods: At first step human foreskin fibroblasts were infected with Retrovirus containing the Yamanaka factors for 24 hours, then they were cultured in a chemically defined medium containing Dorsomorphin, CHIR99021 and SB431542. Results: Induced neural progenitor (iNP) colonies appeared as early as day 13. Many of colonies which stained positive for PAX6 and NESTIN and can be differentiate into mature and functional neurons that express TUJ1 and MAP2. Conclusion: The ability to generate defined, induced neurons from human fibroblasts under a defined condition with predictable fate choices will facilitate disease modeling and therapeutic development. Keywords: Human Fibroblast, Transdifferentiation, Neural Cells

Ps-115: Comparative Studies of Native Human Tyrosinase and Its Two Mutational Variants in HEK-293 Cells

Shahrisa A¹*, Behzadi R², Nikkhah M³, Sadeghizadeh M¹

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Biology, Faculty of Basic Sciences, Islamic Azad University Central Tehran Branch, Tehran, Iran
3. Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Objective: Pigmentation is one of the most obvious phenotypical characteristics in the natural world. Of the pigments, melanin is one of the most widely distributed and is found in bacteria, fungi, plants and animals. Melanins are heterogeneous polyphenol-like biopolymers with a complex structure and color varying from yellow to black. In the melanin formation pathway, tyrosinase is the rate-limiting enzyme that catalyzes tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and oxidizes DOPA to do-paquinone. Tyrosinases belong to type 3 copper proteins. The binuclear active site of tyrosinase consists of two copper ions, coordinated each by three highly conserved histidines. It has been shown that melanin biosynthesis pathway can be feasibly induced using recombinant tyrosinase. Our main aim to evaluate the activity of native tyrosinase and its two mutational variants in HEK-293 cells.

Materials and Methods: Two mutants of human tyrosinase enzyme have been previously constructed in our lab anticipated to possess higher catalytic activity in HEK-293 cells in comparison with the native protein. Native enzyme and the mutational variants have already been cloned in pET-28b(+) for evaluation of protein expression in Escherichia coli BL21. In this approach, coding sequences of native and mutant proteins have been subcloned into pcDNA3.1(+) expression vector followed by subsequent transfection of HEK-293 cells with three constructed pcDNA3.1(+) hTyr vectors.

Results: Digestion and sequencing results of constructed vectors all together showing that fragments have been cloned correctly. transfection's positive control also confirms transformation accuracy. Activity studies of native tyrosinase and two mutational variants have been performed and will be presented in congress.

Conclusion: Human native tyrosinase and two mutational variants have been subcloned from a prokaryotic vector pET-28b(+) into pcDNA3.1(+) for activity evaluation in eukaryotic cells.

Keywords: Subcloning, Expression, Tyrosinase, Mutational Variants, HEK-293 Cell Line

Ps-116: Role of Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) in Gibbon-Ape-Leukemia Virus (GALV) Transduction of CD34+ Cells

Shahrokhi S¹*, Alimoghaddam K², Ghavamzadeh A²

1. Department of Immunoglogy, Faculty of Medicine, Lorestan University of Medical Sciences, Khorram Abad, Iran
2. Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran

Objective: Optimization of transduction condition is an important goal to improve gene transduction. Therefore, we aimed to assess the effect of SP and/or CGRP as novel growth/transducing factors on the efficacy of CD34+ transduction.

Materials and Methods: CD34+ cells were transduced with Gibbon-Ape-Leukemia virus (GALV), containing Neomycin gene. CD34+ cells were transduced with GALV presence of SP and/or CGRP. Real Time PCR and colony formation assay (CFU-C) was performed.

Results: Viral vectors titration on Hela cells indicated transduction efficiency of 1×10⁶ CFU/ml. Real Time PCR of Neo and CFU-C showed stimulatory role of SP on gene transfer 5.9 and 7.86% respectively. CGRP showed 12.58% in control group, while opposite role observed for CGRP 0.89 and 7.86%. Both SP and CGRP showed no significant effect in these assays.

Conclusion: This study showed including of SP in growth factor cocktail is beneficial for CD34+ transduction, which could be applied to genetic modification procedures.

Keywords: CD34+ Cell, CGRP, Neomycin Gene, Retroviral gene transduction, SP

Ps-117: Identification A Novel Regulatory Mechanism Governing One of The Key Stem Cell Pluripotency Genes, SOX2, Mediated by The Non-Coding RNA SOX2OT

Shahryari AR¹*, Malakootian M², Shafiee M³, Vasei M⁴, Mowla SJ¹,²

1. ParsGenome Company, Tehran, Iran

Objective: Optimization of transduction condition is an important goal to improve gene transduction. Therefore, we aimed to assess the effect of SP and/or CGRP as novel growth/transducing factors on the efficacy of CD34+ transduction.
Objective: Long noncoding RNAs (lncRNAs) have emerged as new regulators of stem cell pluripotency and neurogenesis. Interestingly, the SOX2 gene, a master regulator of pluripotency and neurogenesis, is embedded within the 3rd intron of an lncRNA, known as SOX2 overlapping transcript (SOX2OT). SOX2DOT (SOX2 distal overlapping transcript) is an isoform of SOX2OT transcribed from a region located >550 kb upstream of SOX2 is expressed in brain Here, we investigated a potential function of SOX2OT in U87-MG (derived from a glioma tumor) and NT2 cells (a human embryonic carcinoma stem cells) which highly expressed SOX2OT and SOX2.

Materials and Methods: To explore the hypothesis that SOX2OT has a regulatory role on SOX2 expression, we examined the effect of knocking down SOX2OT in NT2 and U87-MG cells and following that, gene expression and cell cycle alterations were examined. NT2 cells were treated with all-trans retinoic acid (ATRA) during four weeks to induce its differentiation into the neuron-like cells. We then evaluated all ESTs of SOX2OT depositing in GenBank implying for existing of several potential splice variants of SOX2OT.

Results: We showed that the lncRNA SOX2OT is a nuclear RNA, and its suppression caused a significant declination of SOX2 expression (0.1, p<0.01) and also induction of G1 cell cycle arrest and prohibition of S-phase entry in NT2 cells. Using different set of primers we identified several novel splice variants of SOX2OT and SOX2DOT that expressed in NT2 stem cells and U87-MG cells. SOX2OT and SOX2DOT variants revealed controversial expression patterns during the course of differentiation of NT2 cells, suggesting their different potential functional links to the undifferentiated and differentiated state of the NT2 cells respectively.

Conclusion: All together, our data suggest a part for SOX2OT and SOX2DOT variants in self-renewal and differentiation of pluripotent stem cells, and a novel regulatory mechanism of SOX2 expression mediated by the lncRNA SOX2OT.

Keywords: SOX2OT, Novel Splice Variants, SOX2, Stem Cells, Differentiation

Ps-118: Expression of Stem and Progenitor Cell Markers in A Spontaneously Arising Human Retinal Pigment Epithelial Cell Line

Shams Najafabadi H*, Soheili ZS1, Samiei SH2, Ahmadieh H3
1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Iranian Blood Transfusion Organization research center, Tehran, Iran
3. Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Email: sjmowla@modares.ac.ir

Objective: RPE is a monolayer of cells located between vessels of the choriocapillaris and light-sensitive outer segments of the photoreceptors. During the embryonic development of the eye, retinal stem cells in the optic vesicle give rise to multipotent progenitor cells that generate all the neurons and the Müller glia of the mature retina. This study describes morphology, viability, growth and characterization of a spontaneously arising human RPE (hRPE) cell line that have been found in our routine hRPE cell culture.

Materials and Methods: RPE was isolated from neonatal human globes and cultured in DMEM/F12 supplemented with 10% FBS. Cultures were continuously studied using phase contrast microscopy. After the 9th passage cultured cells were characterized through immunocytochemistry analysis for Oct4, Chx10, and Pax6 and Ki67 markers.

Results: The first few passages of the cultures were purely RPE cells, those revealed usual morphological features of the pigmented epithelium. Then suddenly alternative variant of cells appeared. They made sizeable holes between RPE cells’ monolayer and push surround of this holes. They finally push all of the culture vessels and RPE cells’ features totally disappeared. The new founded cells were smaller than RPE cells and showed an appreciable tendency to form giant macroscopic colonies. After sequential passages they got smaller than before with a high nucleocytoplasmic ratio, representing thin layer of cytoplasm around the nucleus. The aforementioned cultures were passaged for 13 times until now. They grew faster than native RPE cells and rapidly covered culture surface. When we assessed for viability they showed more than 99% viable. Immunocytochemistry test revealed that 100% of the obtained cells expressed Chx10 whereas just 4% were pax6+. 60% of the studied nuclei were Ki67+ and 50% expressed Oct4.

Conclusion: Our human RPE cultures gave rise to a cell line of hRPE that exhibited retinal cell stemness markers.

Keywords: RPE Cell Line, Oct4, Pax6, Chx10, Ki67

Ps-119: Viability, Growth and Morphology of A Spontaneously Arising Human Retinal Pigment Epithelial Cell Line Those Express Stem/progenitor Cell Markers on Thin Layer of Alginate

Shams Najafabadi H1*, Soheili ZS1, Samiei SH2, Ahmadieh H1
1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Iranian Blood Transfusion Organization Research Center, Tehran, Iran
3. Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Email: shams.hoda250@gmail.com
**Objective:** Retinal pigment epithelium (RPE) is a highly polarized and specialized monolayer epithelium that placed at the interface between the photoreceptors of the neural retina and the vascular layer of the choroid. Almost all cells in the human body grow within organized 3D matrices. Alginates are naturally derived polysaccharides that, is an ideal bioMateriels for tissue engineering. Culturing cells on alginate as a substrate provides a more in vivo-like environment. A spontaneously derived line of human RPE (hRPE) was found in our routine hRPE cultures. It revealed reasonable expression of stem/progenitor cell markers. We cultured the aforesaid hRPE line on alginate films and its apparent characteristics were investigated.

**Materials and Methods:** 940 µl of 1% and 2% (v/w) alginate in deionized water were added in every well of 6 well micro plates for preparing a 1mm alginate film. Alginate substrate was gelatinized using 1X and 10X neurobasal cell culture medium (NCCM). hRPE cell line was isolated from neonatal human globes and cultured in DMEM/F12 supplement with 10% FBS. We characterized these cultures through immunocytochemistry analysis for Oct4, Chx10, Pax6 and Ki67 markers. Cell were cultivated at a density of 200000 on each well of alginate coated 6 well micro plates. After 5 days RPE colonies were harvested and re-cultured on polystyrene substrates.

**Results:** HRPE cell line survived for more than ten days on alginate film. Viability of the cultures was 99%. They grew very well on alginate films; total number of cells increased eleven times respect to controls. The cells formed big sticky colonies on alginate and exhibited bigger colonies on 2% (v/w) alginate gelatinized with 10X (NCCM). When we harvested hRPE colonies and re-cultured them on polystyrene, cells did not switch their morphologic features and maintained their spheroidal structure.

**Conclusion:** We found that alginate can support survival and growth of hRPE cell line and force them to organize in giant colony structures like spheroids. We are going to characterize hRPE cultures more precisely and investigate their differentiating potential into other retinal cells.

**Keywords:** RPE Cell Line, Stem/Progenitor Cell Marker, Alginate, NCCM

**Ps-120:** Amniotic Membrane Extract Induces Limbal Stem Cell Proliferation and Accelerates Corneal Epithelium Healing

Shayan Asl N1*, Jadidi Kh2, Nejat F3, Mohammadi P1, Nekoukar AH2, Ebrahimi M1

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Research and Education Unit, Bina Eye Hospital, Tehran, Iran
3. Animal Core Facility at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Tehran, Iran

**Corresponding Email:** mebrahimi@royaninstitute.org

**Objective:** Corneal epithelium healing in corneal defects is due to limbal stem cells (LSCs) proliferation; so finding a drug for acceleration of these cells growth, is remarkable. In this study we are going to evaluate the effect of amniotic membrane extract (AME) on LSCs proliferation and finally corneal epithelium healing in rabbit model.

**Materials and Methods:** The total protein concentration of AME was determined by Bradford assay. The quantitative amount of EGF, HGF, and KGF was detected by ELISA. Human LSCs, were cultured with different doses of AME (0.1-1 mg/ml) for 14 continues days to assess effective and cytotoxic dose. The LSC and corneal differentiation genes were assessed by Real Time PCR. 10 rabbit were selected and the corneal epithelium mechanically was removed in first day of experiment, then the left eye of each rabbit was treated with 1 mg/ml of AME. The right eye was as control group that were treated only with antibiotics. The Rabbits eyes were observed daily and scored by ophthalmologists. Finally after 2 weeks, 1 month and 3 months post exam were, biopsies from each eye were sent to Iranian Eye Bank for pathological investigation.

**Results:** Our results determined that AME at concentration of 0.1-1 mg/ml induced LSC proliferation (p<0.05). Moreover cells grown in presence of 0.1 and 1 mg/ml AME up-regulated ABCG2 and P63, which are LSCs markers and down-regulated K3, as corneal differentiation markers in compare with cells grown in presence of 10% fetal bovine serum (FBS) (p<0.001). EGF because of having the lowest concentration variation compared to KGF and HGF was determined as the reliable marker for extract quality. Interestingly, in rabbits which were treated with AME, the time of epithelium healing decreased to 2-3 days with lower Edema, chemosis and injection in compare to rabbits in control group. No sign of pathologic abnormalities were observed in test and control groups in vivo.

**Conclusion:** Our results suggest that amniotic membrane extract increase the LSCs proliferation without any differentiation and accelerate the corneal epithelium healing.

**Keywords:** Amniotic Membrane Extraction, Corneal Epithelium, Limbal Stem Cell, Proliferation, Rabbit

**Ps-121:** Expression of TGF-βRI and TGF-βRII Genes in Patients with Multiple Sclerosis following Vitamin D Supplementation

Shirvani Farsani Z1*, Behmanesh M1, Sahraian MA2,3

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Neurology, School of Medical Science, Tehran University of Medical Sciences, Tehran, Iran
3. Multiple Sclerosis Research Center, Tehran University of Medical Sciences, Tehran, Iran

**Corresponding Email:** z.shirvani@gmail.com

**Objective:** Multiple sclerosis (MS) is a complex autoimmune disease of the central nervous system characterized by chronic inflammation, demyelination, and axonal damage. Despite decades of research, the etiology and...
Objective: Prostate cancer that has progressed to metastatic disease remains largely untreatable. Most solid tumors originate from rare tumor cells which are responsible for initiation, progression and tumor metastasis are named cancer stem (like) cells and they may be attractive targets for cancer therapy. Mounting evidence has demonstrated that aggressive and metastatic cells share molecular characteristics with cancer stem like cells such as CD44, a2ß1 integrin and CD133. In this study, we aimed to find whether there is any correlation between stem-cell-related functions and expression level of stemness and metastasis genes.

Materials and Methods: Prostate cancer stem-like cells were isolated from PC3 cell line by spheroid formation as well as expression detection of some surface markers (CD44, CD49b) using BD FACS Aria II. To characterize the sorted cells, doubling time, colony formation potential and sphere formation were done on each group and also the parental line. Quantitative RT-PCR was used to assess the expression level of stemness genes (OCT4, SOX2, NANOG, c-MYC, KLF4) and metastasis genes (CDH1 and CDH2) between tested groups (CD44+/CD49bhi, CD44-/CD49bhi, CD44+/CD49bhi and spheres), and parental cells.

Results: PC3 line, CD44+/CD49bhi and CD44-/CD49bhi formed three different phenotypic clones of colonies are named holo, mero and para clones. The number of holo and mero clones were higher in CD44+/CD49bhi cells compared to CD44-/CD49bhi group (30.2 vs. 2.67% and 21.95 vs. 2.81%); whereas the number of paraclones were higher in CD44- cells (3.8 vs. 2.3%). The proliferation rate and spheroid formation efficiency were higher in PC3 cells (32 hours/ 0.46%) and CD44+/CD49bhi (29.65 hours/ 0.55%) compared to CD44-/CD49bhi group (49.18 hours/ 0.04%), respectively. There was a significant increase in the expression of c-MYC in CD44+/CD49bhi group and KLF4 in CD44-/CD49bhi groups. The expression of CDH1 was significantly increased in spheroid and CD44+/CD49bhi cells compared to the CD44-/CD49bhi ones.

Conclusion: Our data revealed that the subpopulation of CD44+/CD49bhi cells had cancer stem-like cell properties with higher expression of CDH1, therefore we suggest that the E-cadherin, CD44 and CD49b can be considered as suitable markers for identification and isolation of prostate cancer stem-like cells.

Keywords: Prostate Cancer, Spheroid, Stemness, Metastasis Genes

Ps-123: CDH1, CD44 and CD49b As Markers for Identification and Isolation of Prostate Cancer Stem-Like Cells

Shokraie F1,2*, Moharrami M3, Shahhoseini M4, Ebrahimi M2

1. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Molecular and Cellular Biology, University of Tehran, Tehran, Iran
4. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Corresponding Email: marzieh.ebrahimi@gmail.com

Objective: Multiple sclerosis is still unknown, though both genetic and environmental components of the disease seem evident. One potential environmental factor affecting the development of MS may be vitamin D [1,25-(OH)2D3]. A protective effect of vitamin D on multiple sclerosis is supported by the reduced risk associated with sun exposure and use of Vitamin D supplements. Moreover, high circulating levels of Vitamin D have been associated with lower risk of multiple sclerosis. Here we determined whether vitamin D supplementation of MS patients regulated several vitamin D targets. The effect of short-term vitamin D supplementation on selected anti-inflammatory cytokines was assessed in a group of MS patients.

Materials and Methods: To test this hypothesis, Multiple sclerosis patients placed into a vitamin D supplemented group. Blood samples from 10 patients with MS before and 8 weeks after vitamin D therapy. Level of the TGF-β RI and TGF-β RII mRNA measured using Real Time PCR and the results compared.

Results: mRNA levels of TGF-β RI and TGF-β RII increased following vitamin D supplementation.

Conclusion: This is the first study to report the expression of the TGF-β RI and TGF-β RII genes in MS patients supplemented vitamin D. This study further implicates vitamin D as a strong environmental candidate in MS by demonstrating functional interaction with the effecting genes in MS.

Keywords: Multiple Sclerosis, Vitamin D, TGF-β RI, TGF-β RII
Ps-124: Transdifferentiation of Human Adipose Tissue-Derived Stem Cells to Dopaminergic Neurons

Soheilifar MH*, Fakhr Taha M, Javeri A

Department of NanobioMaterials and Tissue Engineering, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Corresponding Email: mftaha@nigeb.ac.ir

Objective: Adipose tissue-derived stem cells (ADSCs) have the capability of differentiation to mesodermal, endodermal and ectodermal lineages. Several studies have demonstrated the differentiation of ADSCs to neuronal and glial phenotypes. The aim of present study was to investigate dopaminergic differentiation of ADSCs in vitro.

Materials and Methods: Human ADSCs were isolated from the adipose tissues collected from patients who underwent abdominoplasty. ADSCs were characterized by flow cytometric analysis. For dopaminergic differentiation, we used a cocktail of sonic hedgehog (SHH) and fibroblast growth factors under a low serum condition. As control, ADSCs were cultured in low serum medium without induction cocktail. After 12 days of differentiation, study of gene expression was performed by RT-PCR and quantitative real-time RT-PCR (qPCR). Immunocytochemistry was used to assess the expression of dopaminergic marker, tyrosine hydroxylase (TH), and mature neuronal marker, neurofilament light polypeptide (NEFL).

Results: Flow cytometric analysis confirmed the mesenchymal nature of the ADSCs. RT-PCR study showed the expression of dopaminergic neuron-specific-genes, including TH, EN1, NURR1 and PITX3 mRNAs, in the treatment group. The control group only showed the expression of EN1 and NURR1 mRNAs. QPCR analysis demonstrated upregulation of TH and NURR1 mRNAs in the treatment group compared to control group. Immunostaining revealed the expression of TH and NEFL proteins in the differentiated ADSCs.

Conclusion: Adipose tissue is an abundant source of adult stem cells with the potential for differentiation to dopaminergic neurons. ADSCs might be a valuable source for the future cell replacement therapy especially in regards to disorders involving loss of dopaminergic neurons.

Keywords: Adipose Tissue-Derived Stem Cells, Differentiation, Dopaminergic Neuron

Ps-125: PPARγ Agonists Treatment Did Not Significantly Enhance The Expression Levels of Neural Precursor Cell Markers during Neural Differentiation of Human Embryonic Stem Cells

Taheri M1, 2*, Salamian A2, Ghaedi K2, 3, Nasr Esfahani MH2, Baharvand H4, 5

1. Department of Biology, Islamic Azad University (IAU), Sciences and Research Branch, Tehran, Iran
2. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
4. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Biology and Technology, ACECR, Tehran, Iran
5. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Corresponding Email: kamranghaedi@yahoo.com

Objective: Human embryonic stem cells (hESCs) are pluripotent cells that have the potential to differentiate into various cell types such as neural differentiation. PPARγ (peroxisome proliferator-activated receptor gamma) is a member of the nuclear receptors, which exerts various metabolic functions including differentiation process. Moreover, several natural and synthetic compounds are categorized as PPARγ agonists such as 15d-PGJ2 and Pioglitazone acting by binding to PPARγ and active its transcriptional activity. The aim of the present study was evaluating the effect of PPARγ agonist on human neural precursor cells (NPCs) formation in vitro.

Materials and Methods: Human ESCs were differentiated into the neural lineage in defined adherent culture. hESCs were incubated with basic fibroblast growth factor (bFGF) and noggin for 4 days, then cells differentiated into neuroectodermal cells with retinoic acid (RA) for 6 days. Columnar cells were beginning to organize into rosettes. The cells were simultaneously treated with RA and PPARγ agonists, 15d-PGJ2 and Pioglitazone. Treated cells with Pioglitazone were collected in this time and those were treated with 15d-PGJ2 were cultured without RA for more six days in the same media. Real-time PCR was performed for NPCs markers including NESTIN, SOX1, and PAX6.

Results: Neuroectodermal cells within the neural tube were characterized by expression of several markers including NESTIN, SOX1 and PAX6. Data were evident that treatment with 15d-PGJ2 and Pioglitazone caused...
insignificant difference in rosette structure formation known as neural precursor cells. To seek additional evidence, real-time PCR analyses were performed which were shown a trivial rising in the mentioned NPCs markers.

**Conclusion:** Collectively, our data indicated that PPARγ activation via treatment with 15d-PGJ2 and Pioglitazone during NPCs formation caused insignificant difference expression of neural precursor markers. Importantly, these results are in concordance with our previous data regarding the role of PPARγ during neural differentiation of mouse ESCs.

**Keywords:** Human Embryonic Stem Cells, Neural Precursor Cells, PPARγ, Real-time PCR

**Ps-126: Establishment and Biological Evaluation of The Goat Fetal Fibroblast Cell Line**

Tajeldini M1, Mehrabani D2*, Tamadon A1, 2, Dianatpour M3, 4, Parvin F2, Rahmanifar F4

1. Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
2. Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

**Corresponding Email:** davood_mehrabani@yahoo.com

**Objective:** Fibroblast cell lines have different application in biological research including nuclear transfer in cloning, feeder layer of embryonic stem cells, wound healing research and tissue engineering. The objective of the present study was to establish and biological evaluation of goat fetal fibroblast before and after cryopreservation.

**Materials and Methods:** Goats fetus (age about 51, 53 and 55d) were collected from Shiraz Slaughterhouse. Their skin was cut into small pieces (1 mm3) and cultured in DMEM and FBS. When reaching 80-90% confluence, the cells were passaged. Cells of the 8th passage were cultured in 24-well plates (1.5×105 cells/well) for 9 days after freezing. P3Ts were 59.7 hours on day 51, 90.6 hours on day 53, and 53.1 hours in day 55 of pregnancy. After freezing, PDTs were 54.6 hours on day 51, 52.1 hours on day 53, and 65.5 hours on day 55 of pregnancy. The freezing had a little effect on the viability of the cells.

**Results:** The goat fetal fibroblasts after passage had a latent phase of about 48 hours. After an exponential phase, cells entered the plateau phase on day 5. Before freezing, PDTs were 59.7 hours on day 51, 90.6 hours on day 53, and 53.1 hours in day 55 of pregnancy. After freezing, PDTs were 54.6 hours on day 51, 52.1 hours on day 53, and 65.5 hours on day 55 of pregnancy. The freezing had a little effect on the viability of the cells.

**Conclusion:** The goat fetal fibroblast line was established using the adherent culture method and cryopreservation. After freezing, growth and viability indices of the cells were favorable.

**Keywords:** Cell Line, Fibroblast, Growth Curve, Cryopreservation, Fetus, Goat

**Ps-127: Histological Analysis of Bone Repair in Rat Femur via Nanostructured Merwinite Granules**

Talebi AR1*, Hafezi M2

1. Research and Clinical Center for Infertility, Department of Anatomy, Shahid Sadsoughi University of Medical Sciences, Yazd, Iran
2. BioMaterials Group, Nanotechnology & Advanced Materials and Energy Research Center, Tehran, Iran

**Corresponding Email:** prof_talebi@ssu.ac.ir

**Objective:** There is a conspicuous need for in vivo evaluation of biomaterials prior to their application as bone graft or tissue engineering scaffold. Merwanite and HA in vivo application as bone filler would be studied in this study.

**Materials and Methods:** Femoral injury was induced in male rats and early and late degrees in bone repair were evaluated by histochemical stainings.

**Results:** A histomorphological analysis of the bioceramics implants in rat femoral defect models suggested that both in early and late stage of bone repair, merwanite is more effective in promoting osteogenesis in comparison with HA and in late stage, the rate of new bone formation was faster in merwanite-filled-bone-defect than in HA models. The control groups showed limited osteogenesis.

**Conclusion:** These results suggested that merwanite might be a potential and attractive bioceramic for bone replacement.

**Keywords:** Bioceramic, Merwanite, In Vivo, Histological Analysis

**Ps-128: Valproic Acid, A HDAC1 Inhibitor, Greatly Reduces Lesion Formation and Ameliorates Locomotor Function in Rat Spinal Cord Injury**

Tiraihi T1, Abdanipour A2*, Scheluesener H3

1. Division of Immunopathology of the Nervous System, Tuebingen University, Tuebingen University, Tuebingen, Germany
2. Stem Cells Research Laboratory, Department of Medical Science, Ardabil Branch, Islamic Azad University, Ardabil, Iran
3. Department of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

**Corresponding Email:** abdani.anatomy@yahoo.com

**Objective:** Valproic acid (VPA) is a histone deacetylase (HDAC1) inhibitor. Acetylation of histones is critical to cellular inflammatory and repair processes.

**Materials and Methods:** In this study, VPA was intraperitoneally administered three hours after injury for 7 days (once a day) at a dose of 400 mg/kg.

**Results:** The results showed a reduction in the develop-
ment of secondary damage in rat spinal cord trauma with an improvement in the open field test (BBB scale) with rapid recovery. VPA administration increased regional BDNF and GDNF mRNA levels.

**Conclusion:** Local inflammation, the expression of the lysosomal marker ED1 by activated macrophages/microglial cells, was reduced by VPA and immunoreactivity of acetylated histone (H3) and microtubule-associated protein (MAP2) increased.

**Keywords:** Spinal Cord Injury, Epigenetic, Valproic Acid, Histone Acetylation

**Ps-129: Expression Alteration of miR-2276 in Tissue Samples Obtained from Patients with Breast Cancer**

Torkashvand S*, Tavallaei M2, Vasei M2, Mirzaei B1, Mowla SJ1

1. Department of Genetics, Basic Science Faculty, Tarbiat Modares University, Tehran, Iran
2. Department of Human Genetics, Human Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Department of Pathology, Pathology Center, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Genetics, Faculty of Biological Sciences, Research Branch, Islamic Azad University, Tehran, Iran

**Corresponding Email:** sjmowla@yahoo.com

**Objective:** Breast cancer is one of the most common malignancies in women. It continues to be a major burden and cause of death among women worldwide. Most patients die of distant metastases that are frequently unresponsive to cancer therapy. In order to metastasize, cells need to be able to migrate and invade into the surrounding tissue, intravasate in to a blood vessel or lymphatic system, survive in circulation, extravasate and finally proliferate at a distant site. Piwi (P-element-induced wimpy testis) is a subgroup of the Argonaute proteins. The genes of the piwi family are defined by conserved PAZ and Piwi domains and play important roles in stem-cell self-renewal, RNA silencing and translational regulation in various organisms. MicroRNAs (miRNAs) are small noncoding RNAs (19 to 24 nucleotides) with regulatory functions, which play an important role in breast cancer. miRNAs can act either as tumor suppressors or as oncogenes, and that measurement of miRNA expression in malignancies may have diagnostic and prognostic implications. Although miR-2276 is target Piwil2 gene and Piwi2 role in stem-cell self-renewal, the molecular pathway of Piwi2 in breast cancer and its role in proliferation of breast cancer cells. This study was designed to evaluate the expression alterations of miR-2276 in breast cancer tissue samples.

**Materials and Methods:** Total RNA was extracted from paraffin-embedded tissue breast cancer samples using trizol. Afterward, cDNA synthesis and Real time-PCR assay were performed by specific stem-loop primers.

**Results:** Results of our study, up to now, demonstrated significant changes of miR-2276 expression in tumor samples in comparison with non-tumor samples.

**Conclusion:** Considering previous and related studies, miR-2276 may have important role in breast cancer pathogenesis among susceptible women. Therefore, this miRNA can be applied as a breast cancer marker in diagnostic and prognostic tests.

**Keywords:** Breast Cancer, miR-2276, Piwil2

**Ps-130: RNA Microarray Analysis Showed Significantly Changed Gene Expression after In Vitro Human Cardiac Stem Cells and Satellite Cells Passages**

Vahdat S1*, Moosavi SA2, Abrari M2, Hajhosseini V2, Gharechahi J2, Hosseini Salekdeh Gh2, Aghdami N1, 3

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Molecular Systems Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Regenerative Biomedicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

**Corresponding Email:** nasser.aghdami@royaninstitute.org

**Objective:** Increasing rate of morbidity and mortality by heart diseases all over the world attracted scientists’ attention to this important medical case. In recent years regenerative medicine has been introduced as an alternative tool for heart repair, and cardiac stem cells (CSCs), precursor cells resident in heart tissue have been investigated as novel sources of heart cell therapy. Such patient derived stem cells need to be isolated from small heart biopsies and expanded in culture to reach the quantity which is required for cell therapy. Although various aspects of adult stem cell transplantation have been well studied, the effects of extensive sub-culturing on the characteristics and phenotype of these cells have remained elusive.

**Materials and Methods:** Human CSCs and skeletal muscle stem cells (satellite cells) were isolated from heart and intercostals biopsies of patients with congenital diseases during routine heart surgeries with informed consents. Isolated cells were passaged until their morphologies began to change and they were sampled at 4 passages -passage 6, 9, 12 and 15. Cell surface markers’ changes were analyzed using flowcytometery system and whole genome transcriptom alteration was assayed using Illumina chip microarray analysis.

**Results:** Doubling time measurement results showed that subsequent passages caused an increase in essential time for cell number duplication and flowcytometry analysis indicated the different cell surface markers at different passages. Whole genome analysis showed gene expression alteration by increasing the passage number (ANOVA p value<0.05, fold change=2). The most important gene ontology which significantly altered during passages was cell cycle.

**Conclusion:** Our study showed that subsequent passages cause significant changes in both phenotypic and
genome expression characteristics of CSCs and satellite cells. As both CSCs and satellite cells are considered as stem cell therapy sources, it’s important to decrease any cell modification in culture.

**Keywords:** Cardiac Stem Cells, Satellite Cells, Microarray Technology, Subsequent Passages

**Ps-131:** Direct Differentiation of Human Pluripotent Stem Cells into Functional Motor Neurons Using Small Molecules

Valizadeh Arshad Z1, 2*, Shahbazi E1, Hashemizadeh Sh1, Baharvand H1, 2, Kiani S1

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Corresponding Email: skiiani2536@yahoo.com

**Objective:** Motor neuron differentiation from human pluripotent stem cells (hPSC) is a goal in regenerative medicine for cell therapy in diseases that lose these cells. Most protocols have low efficiency in generate functional motoneurons, therefore small molecules are new idea for dominance this challenge.

**Materials and Methods:** Under this approach we tried to use an efficient, short time and cost effective protocol than reported protocols to generate functional motoneurons from hPSC. Therefor we used small molecules Dorsomorphin, A8301, XAV, Retinoic acid and Purmorphamine in this protocol.

**Results:** Immunofluorescent staining and flow cytometry analysis of hESC-derived neural ectoderm (NE) showed these structures are 92.68% NESTIN+, 64.40% PAX6+, 82.11% SOX1+ in a chemically defined adherent culture. After replating of hESC-derived NE, the differentiated cells are Tuj1+, MAP2+, HB9+ and ISL1+. level of gene expression evaluated with real time RTPCR in different stages of differentiation protocol. Electrophysiological properties of differentiated cells recorded by whole cell Patch clamp technique. hESC derived motor neurons displayed voltage gated delay rectifier K+ and Na+-Ca2+ inward currents.

**Conclusion:** Our findings suggested that, hESC-derived neurons expressed special motor neuron proteins such as HB9 and ISL1 but electrophysiological properties showed that these cells were not mature because the resting membrane potential was more positive than mature motor neuron and Na+-Ca2+ inward currents were not sufficient for firing action potentials. It seems that the cells should be more developed for functional maturation.

**Keywords:** Pluripotent Stem Cells, Motor Neuron, Small Molecule, Differentiation, Whole Cell Patch Clamp

**Ps-132:** Biomarkers of Endocrine Disruption: Cluster Analysis of Effects of Plasticisers on Phase 1 and Phase 2 Metabolism of Steroids

Waring R1*, Ramsden D2, Jarratt P3, Harris R4

1. Biosciences, University of Birmingham, Birmingham, UK
2. Department of Clinical and Experimental Medicine, University of Birmingham, Birmingham, UK
3. Department of Computer Sciences, University of Birmingham, Birmingham, UK
4. Biosciences, University of Birmingham, Birmingham, UK

Corresponding Email: r.h.waring@bham.ac.uk

**Objective:** Endocrine disruptors (EDs) are environmental compounds which affect steroid synthesis or steroid metabolism and so alter physiological responses. The objective was to design a profile of in vitro tests to identify EDs so that safer industrial compounds could be introduced and much animal testing avoided.

**Materials and Methods:** Plasticisers, including phthalates, alkyl phenols, adipates and other phenols were selected as known EDs and 16 of these compounds were assayed in 23 different tests for steroid metabolism, steroid nuclear receptor action, cell signalling, thyroid metabolism.

**Results:** The matrix of results was analysed by cluster analysis. This showed that 4 of the 23 tests were particularly informative. EDs altered the activity of SULT 1E1, a sulphotransferase enzyme which inactivates oestrogens by sulphation, the provision of inorganic sulphate for oestrogen sulphation, the activity of aromatase for oestrogen synthesis and thyroid metabolism.

**Conclusion:** Both genomic and non-genomic effects of EDs are important. A profile of rapid robust in vitro tests to identify EDs was produced and the ranking on this test was in accordance with the known ED effects of the compounds tested.

**Keywords:** Endocrine Disrupters, Steroids, Biomarkers, Cluster Analysis, Plasticisers

**Ps-133:** Optimization of Buffalo (Bubalus Bubalis) Embryonic Stem Cell Culture System

Zandi M1*, Sanjabi MR1, Khamoushi S1, Chauhan MS2

1. Department of Animal and Poultry Science and Fisheries, Agricultural Institute, Iranian Research Organization for Science and Technology, Tehran, Iran
2. Dairy Extention Division, National Dairy Research Institute, Karnal, India
3. Animal Biotechnology Centre, National Dairy Research Institute, Karnal, India

Corresponding Email: mz1075@yahoo.com

**Objective:** In order to retain an undifferentiated pluripotent state, embryonic stem (ES) cells depend on culture on feeder cell layers. However, use of feeder layers limits stem cell research design, since experimental data may result from a combined ES cells and feeder cell response to various stimuli. In this study, the effects of FGF-2 (5 ng/ml), LIF (1000 U) and Y-27632 (10 μM) in gelatin based culture were analyzed and feeder layer based culture and gelatin coat based culture, either alone or in the presence of feeder-conditioned media (CM) from fibro-
blast cells, were compared. Alkaline phosphatase and immunofluorescence staining (pluripotency and cell surface markers) were used to characterize buffaloes ES cells.

**Materials and Methods:** Data were analyzed by ANOVA, and statistical significance was accepted at p<0.05.

**Results:** The results showed that Y-27632, in presence of FGF-2 and LIF, resulted in higher colony growth and increased expression of NANOG gene. Feeder-CM resulted in a significant increase in growth of buffaloes ES cells on gelatin coated plates, however, feeder layer based culture showed better results than gelatin based culture. Feeder layer from buffalo fetal fibroblast cells support buffaloes ES cells for more than two years.

**Conclusion:** In conclusion we developed a feeder free culture system that support buffaloes ES cells for short term maintenance as well as feeder layer based culture that support long term maintenance of buffaloes ES cells.

**Keywords:** Buffalo, Embryonic Stem Cells, Y-27632, FGF-2, LIF, Gelatin Based Culture

**Ps-134: WNT3A Signaling Pathway Has Significance to Maintain Undifferentiated State of Buffalo Embryonic Stem Cells**

Zandi M*, Sanjabi MR1, Khamoushi S1, Chauhan MS2,3

1. Department of Animal and Poultry Science and Fisheries, Agricultural Institute, Iranian Research Organization for Science and Technology, Tehran, Iran
2. Dairy Extension Division, National Dairy Research Institute, Karnal, India
3. Animal Biotechnology Centre, National Dairy Research Institute, Karnal, India

**Corresponding Email:** mz1075@yahoo.com

**Objective:** The present study was aimed to study the effects of WNT3A signaling pathway on buffalo embryonic stem (ES) cells. WNT proteins play roles in the regulation of gene expression, cell proliferation, and differentiation and in the maintenance of cell polarity.

**Materials and Methods:** Data were analyzed by ANOVA, and statistical significance was accepted at p<0.05.

**Results:** The increase in mean colony area between days 1 and 6 was significantly lower (p<0.05) with 400 ng/mL than that with 0 (control), 100 or 200 ng/mL WNT3A. The increase in mean colony area was significantly higher (p<0.05) in the presence of WNT3A+FGF-2+LIF than in the presence of FGF-2+LIF which, in turn, was higher (p<0.05) than when only WNT3A was present or that in the controls. Real-time qPCR analysis showed that the relative mRNA abundance of β-CATENIN was significantly increased (p<0.05) and that of SOX2, OCT3/4 and C-MYC was not affected by WNT3A supplementation at all the concentrations examined. Both WNT3A and FGF-2+LIF, either alone or in combination, significantly increased (p<0.05) the expression level of β-CATENIN and NANOG compared to that in the control.

**Conclusion:** WNT3A works together with exogenous factors it leads to no significant affect on proliferation of buffaloes ES cells.

**Keywords:** WNT3A, Embryonic Stem Cells, Buffalo
1. Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

Corresponding Email: zarrinhaghighi.a@gmail.com

**Objective:** Over last years, extensive efforts have been made to find new ways for treatment of congenital diseases. One of the most relying approaches is fetal therapy. During embryonic development, organ development will not be completed. Thus, it is an opportunity to treat some congenital diseases before termination of organ development. As regards organ development did not complete in embryonic development, it seems that we can treat some congenital diseases before finishing development of organs.

**Materials and Methods:** Search of the literature published in English using the PubMed database, intellectual comparison and deduction.

**Results:** Fetal therapy contains all of works -medical or surgical- that performed for treatment of sick fetus. In this study, new approaches of regenerative medicine combined to fetal therapy. Recapitulation, stem cell therapy and gene therapy may help developing of fetal therapy as a new way for treatment of congenital diseases. Furthermore, these subjects combined and discussed as a new approaches of fetal therapy.

**Conclusion:** Prenatal diagnosis of congenital diseases can lead to the diagnosis of severe fetal abnormalities. Fetal therapy can be alternated to terminate pregnancy in abnormal fetuses. Fetal gene therapy using stem cells (e.g. hematopoietic stem cell) is a promising new approach. Future development of this technique needs safe protocols and preclinical investigations on animals prior clinical trials in human fetuses. Although fetal therapy may open new horizons for treatment of several diseases, there are major ethical aspects needs to be considered.

**Keywords:** Fetal Therapy, Stem Cell, Gene Therapy, Recapitulation, Fetus, Pregnancy

Ps-137: Differential Expression Level of Nestin in Mesenchymal Stem Cells Derived from Human Chorion and Bone Marrow

Ziadlou R1,2,3*, Sayahpour FA2, Safari F2, Baghaban Eslaminejad M2, Shahhoseini M3

1. Department of Cellular and Molecular Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Corresponding Email: eslam@royaninstitute.org, m.shahhoseini@royaninstitute.org

**Objective:** Nestin is an intermediate filament protein that has been extensively used as a marker for central nervous system (CNS) progenitor cells in different studies. On the other hand, Mesenchymal stem cells (MSCs) are originally known to possess capacities to differentiate into mesenchymal cell lineages including bone, cartilage and adipose cells. Furthermore according to previous studies, Nestin positive MSCs tended to differentiate into neuronal cell lineages *in vitro*. In the search for other suitable source for MSCs, in this study we tried to isolate stem cells from human chorion and compared them to those of bone marrow (BM) in terms of differentiation capacities. Moreover we compared the two cells with respect to the expression level of Nestin which is an indicator of cell potential in differentiating into neural cell lineages.

**Materials and Methods:** Chorionic plate was isolated from patients undergoing cesarean and subjected to the enzymatic digestion using collagenase IV. Released cells were then plated and cultured. Bone marrow was obtained from patients volunteered for cell therapy at Royan Institute. Passaged-3 cells were compared in terms of surface epitopes and multilineage differentiation potential. The expression level of Nestin in BM-MSCs and chorion stem cells (CSCs) were evaluated quantitatively by RT-PCR technique.

**Results:** Immunophenotyping of BM-MSCs and CSCs demonstrated that the majority of both cells were positive for mesenchymal markers (CD105, CD44, CD90, and CD73). Hematopoietic cell lineage markers (CD34 and CD45) were expressed on small percentages of the cells. Both cells succeeded to differentiate into mesenchymal lineages. According to our findings, CSCs showed significantly higher expression level of Nestin in comparison to BM-MSCs (p<0.05).

**Conclusion:** Consequently, since CDSCs have expressed Nestin in high levels, it would be regarded as a source of stem cells appropriate for cellular therapy in neurological disorders.

**Keywords:** Nestin, Human Mesenchymal Stem Cells, Chorion, Bone Marrow
List of Pre-Congress Courses and Workshops

Royan Institute
Cell Science Research Center

Tehran, Islamic Republic of Iran
Mesenchymal Stem Cells: Isolation, Purification and Differentiation
  • **Scientific Manager:** Baghaban Eslaminejad MR., Ph.D.
  • **Executive Manager:** Faani N, M. Sc
  • **Date:** September 1-2, 2013

Semen Analysis
  • **Scientific Manager:** Salman Yazdi R, Ph.D.
  • **Executive Manager:** Sabbaghian M, Ph.D.
  • **Date:** September 2, 2013

Advanced Vitrification Techniques: What to Vitrify and When to Vitrify?
  • **Scientific Managers:** Conaghan J, Ph.D, Spach J, B.Sc, Karimian L, M.Sc
  • **Executive Managers:** Eftekhari Yazdi P, Ph.D, Hadi M, B.Sc
  • **Date:** September 2, 2013

New Perspectives in COS and Embryology
  • **Scientific Managers:** Humaidan P, M.D., Meseguer M, Ph.D., Ashrafi M, M.D.
  • **Executive Manager:** Jahangiri N, M.Sc
  • **Date:** September 3, 2013

Sonographic and Color Doppler in Infertility
  • **Scientific Managers:** Kurjak A, M.D., Ahmadi F, M.D.
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  • **Date:** September 4, 2013
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* MERT Combined Analysis Paper, Plateau et al. 2006, RSM Online : 190-196