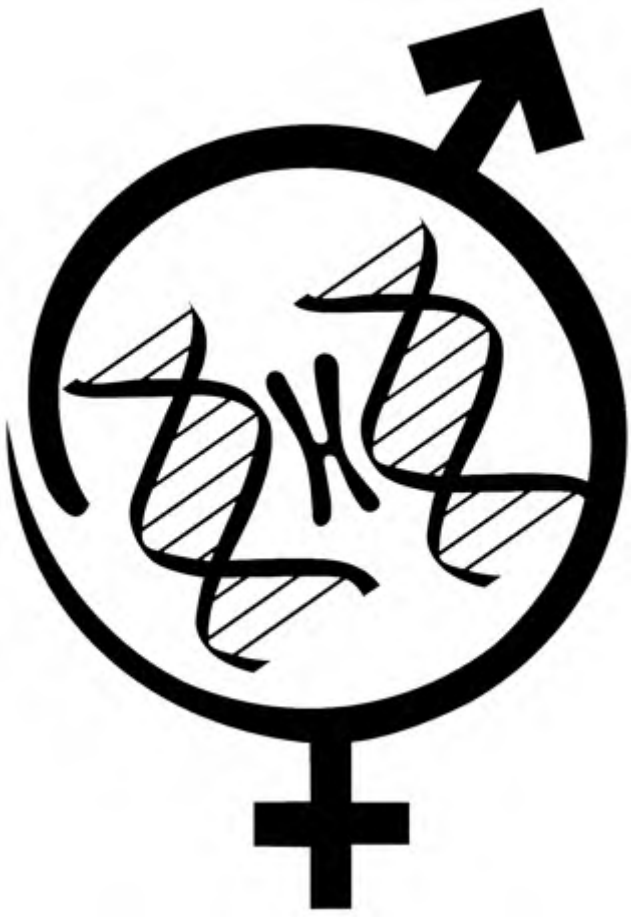
Abstracts of

Royan International Twin Congress

10th Congress on Stem Cell Biology and Technology

3-5 September 2014



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Cell Journal(Yakhteh)

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IN THE NAME OF GOD

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.

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Contents

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Congress Chairman

Mehdi Totonchi

Dear Colleagues and Friends,

On behalf of organizing committee, it is my great pleasure and honor to welcome you to the 10th International Congress on Stem Cell Biology and Technology which is held accompanying with 15th congress on Reproductive Biomedicine (Royan International Twin Congresses), on September 3-5, 2014.

Regarding 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 10th International Congress on Stem Cell Biology and Technology on September 3-5, 2014. 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 science world.

Hope to see you in Tehran, Iran
Mehdi Totonchi, Ph.D.
Congress Chairman
Stem Cell Biology and Technology Congress

Is-1: Past, Present and Future of Planarian Regeneration Research

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Is-2: Endogenous versus Exogenous Approach to Regenerate Intervertebral Disc Damage

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Is-3: Stem Cell Biology and Regenerative Medicine at Royan Institute: Current Status and Future Prospects

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After the first report on the generation of human embryonic stem cells (hESCs) from preimplantation embryos, in the early 2000s, Royan institute had a relatively strong start in the stem cell field that benefited from religious blessings, and scientific endeavors on the part of non-governmental and public research organizations and universities. Recently, the research spanning the spectrum of discovery science, translational research and clinical application, and has focused on developing new ways in developmental biology, nanobiotechnology and tissue-engineering, transdifferentiation, and small-molecule modulation in regenerative medicine. In parallel with progress made in medical and stem cell research, required infrastructures for cell therapy such as Good Manufacturing Practice facility has been developed in the last few years for clinical trials in patients with myocardial infarction, vascular, liver, skin, eye, bone, cartilage, and neurological disorders with autologous cell transplantation. I will review the current status and our perspectives for stem cell biology and regenerative medicine.

Is-4: Role of Endogenous Neural Precursor Cells in Multiple Sclerosis

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Objective: In the adult brain, microenvironments that maintain a source of neural stem cells and NPCs are the SVZ of the lateral ventricle and the DG of the hippocampus. Experimental demyelination and MS, reactivate the SVZ, leading to increased proliferation, oligodendrogenesis and ectopic migration to periventricular white matter. The goal of this paper is to review the importance of specialized cellular niches showing plasticity regarding cell number, fate decision, migration and differentiation in the context of demyelinating pathologies such as multiple sclerosis (MS).
Materials and Methods: Various tracing paradigms in combination with stem cell markers have allowed to study reactivation of the adult stem cell niches in a number of disease animal models going from rodents to non-human primates and MS tissues.
Results: Tracing studies indicate that while -B cells are the SVZ source of newly formed oligodendrocytes in normal conditions, -A and -C cells undergo a functional switch in response to demyelination to increase oligodendrogenesis. Specialized cell-cell interactions occur within the niche, suggesting ongoing communication within the different cell types and namely specialized interactions between ependymal cells and B cells. Gaining more insights in the modulation of these interactions in animal models, could help to understand how the niche is reactivated in response to inflammatory demyelination. Maintaining the balance between neural stem cells and NPCs in the SVZ niche is critical to supply the brain with specific neural populations, both under normal or demyelinating conditions. Several factors influencing SVZ homeostasis were elucidated and found to play a crucial role in regulating the balance between neural stem cells and NPCs as well as neurogenic and oligodendrogenic NPCs. Some of these mechanisms are perturbed in response to chronic inflammation leading to enhanced SVZ thickness, increased oligodendrogenesis at the expense of neurogenesis. Furthermore, -A and -B cells are vulnerable to chronic inflammation, further contributing to a diminished neuronal renewal in the OBs and resulting in olfactory deficits. A major question concerns the nature of the molecular cues involved in the correct targeting of the migrating NPCs to the OBs. These include growth factors, cell adhesion molecules, integrins, chemokines and repellents. While, growth factors and chemokines were identified as major players in ectopic recruitment of SVZ cells to lesion sites, little is known on the role of other molecular cues in directing NPCs and derivatives to the lesion site.
Conclusion: In spite of the demonstration of the adult SVZ reactivation in experimental models and MS, mobilization, differentiation and oligodendrocyte recruitment remain limited. Therefore, understanding the mechanisms regulating the biology of the stem cell niches in normal and pathological conditions could help in designing therapeutic strategies preventing the loss of NPCs and promoting their proliferation, migration and differentiation for myelin diseases such as MS.

Keywords: Adult Neural Stem Cells, Oligodendrogenesis, Myelin, Demyelination, Remyelination

Is-5: Human Pluripotent Stem Cells for Modelling and Correcting Long-QT Syndrome

Bellin M[1, 2]*, Moretti A[2],Casini S[1],Welling A[3], Zhang H[4], Zou B[4], Li M[4], Laugwitz KL[2], Mummery C[1]
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Objective: Long-QT syndrome (LQTS) is an electrical disease of the heart characterized by delayed cardiac repolarization, which causes prolongation of the QT interval (the distance between the Q and T peaks) on the surface electrocardiogram. Its clinical manifestations are often dramatic, with ventricular tachycardia and syncope resulting in cardiac arrest and sudden death. The LQTS variants LQT1, LQT2, and LQT3 comprise the majority documented to date. The objectives of our studies were 1) to demonstrate that human induced pluripotent stem cells (hiPSCs) are a suitable tool to recapitulate the LQT1 phenotype by studying the R190Q-KCNQ1 mutation; 2) to investigate whether elevation of the intact repolarising current IKr could effectively restore normal QT duration if KCNQ1 is mutated; 3) to study the LQT2-associated N996I-KCNH2 mutation under genetically defined conditions, by generating two genetically distinct isogenic pairs of LQT and control lines.
Materials and Methods: We reprogrammed skin fibroblasts from patients affected by LQT1 and LQT2 syndrome and healthy controls. The generated hiPSCs were differentiated into cardiomyocytes (CMs) and their electrophysiological properties were studied with the patch-clamp technique. Gene targeting based on homologous recombination was used to correct LQT2 hiPSCs and introduce the same KCNH2 mutation in human embryonic stem cells (hESCs).
Results: We generated patient-specific hiPSCs from members of a family affected by LQT1 and differentiated into functional CMs. These cells recapitulated the electrophysiological features of the disorder, including prolongation of the action potential duration (APD), as compared with cells from healthy controls. Further characterization of the role of the R190Q-KCNQ1 mutation in the disease pathogenesis revealed a dominant negative trafficking defect associated with a 70% to 80% reduction in the KCNQ1-conducted IKs current and altered channel activation and deactivation properties. Furthermore, mutated CMs had an increased susceptibility to catecholamine-induced tachyarrhythmia and beta-blockade attenuated this phenotype. Next, a unique specific chemical activator for IKr that reduced voltage sensitivity of inactivation, caused a dose-dependent shortening of the APD and was able to normalize action potentials of CMs of patients with LQT1. Finally, we derived hiPSCs from a patient carrying the LQT2-associated N996I-KCNH2 mutation and corrected it. Furthermore, we introduced the same mutation in hESCs, generating two genetically distinct isogenic pairs of LQTS and control lines. Correction of the mutation normalized the IKr current conducted by the KCNH2 channel and the APD in hiPSC-derived CMs. Introduction of the same mutation reduced IKr and prolonged the APD in hESC-derived CMs. Further characterization of N996I-KCNH2 pathogenesis revealed a trafficking defect.
Conclusion: Our results demonstrate that hiPSC models recapitulate aspects of genetic cardiac diseases, including APD prolongation, altered IKs, and an abnormal response to catecholamine stimulation, with a protective effect of beta-blockade. Furthermore our studies provide both the theoretical basis and experimental support for compensatory normalization of APD by a pharmacological agent. Finally we have demonstrated that the N996I-KCNH2 mutation is the primary cause of the LQTS phenotype. Precise genetic modification of pluripotent stem cells provided a physiologically and functionally relevant human cellular context to reveal the pathogenic mechanism underlying this specific disease phenotype.
Keywords: Long-QT Syndrome, Human Pluripotent Stem Cells, KCNQ1, KCNH2, Gene Targeting

Is-6: Bioengineer Attempts to (Re-) Generate New Nephrons and The Kidney

Benigni A

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Chronic kidney disease (CKD) is a global public health problem whose prevalence has been estimated to be 8-16% of the population worldwide. Eventually end-stage renal disease (ESRD) occurs every year in 0.15-0.2% of patients with overt CKD, and renal replacement therapy (RRT) with dialysis or transplantation is required. Although approximately 2 million people are currently on RRT to sustain life worldwide, this likely represents less than 10% of those who need it. The kidney transplant approach is also seriously impaired by eventually limited graft survival and by the scarce availability of donors. Innovative tissue engineering strategies have been recently proposed to overcome these unmet needs.
Recent advances in our understanding of the developmental biology of the kidney, along with the establishment of novel methodologies in the field of regenerative medicine, have created significant potential for kidney

regeneration. These advances include construction of novel kidney tissue from suspensions of single cells of metanephric origin. Another innovative tool under current investigation rests on the idea of using decellularized kidneys for engineering a new functional organ as potential future treatment option for ESRD. In the last five years several interesting observations have been reported on the possibility to used an acellular matrix from the whole kidney and the attempt to recellularize this scaffold using stem or differentiated cells.
Despite difficulties in achieving the important goal of kidney engineering into the laboratory, the currently tested methodologies are offering new chances to possibly regenerate the kidney from patients with CKD in the near future.

Is-7: Mesenchymal Stem Cells as A Cure for Acute Kidney Injury

Benigni A

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Acute kidney injury (AKI) is emerging as a public health problem in developing and developed country that affects up to 7% of hospitalized patients with higher prevalence in critical care unit. Despite major advances in preventive strategies and support measures, the mortality rate among patients remains higher than 50%. Several pharmacological approaches to improve renal function and survival after AKI episode have been largely unsuccessful in clinical practice. New hopes of innovative interventions to enhance the limited capability of kidney regeneration in AKI comes from stem cell-based therapy with mesenchymal stromal/stem cells (MSC) that represent an attractive tool by virtue of their unique properties, of homing damaged tissues and the regenerative capacity.
Tubular epithelial cells that after acute ischemic or toxic insults undergo dysfunction and detachment and are key target for cell therapy in AKI. MSC have been proved effective in curing experimental AKI. The mechanisms underlying the reno-protective effect in preclinical models of AKI has been evaluated, together with modalities by which MSC interact with damaged cells via release of soluble factors and exosomes/microvescicles. Several biological effects of the identified mediator will be analyzed in the lecture including anti-apoptotic, promitogenic, immunomodulatory and anti-inflammatory activities. The mechanisms of stem cell homing and engraftment to sites of tissue damage will be discussed.
Conclusion: Translation of preclinical data with stem cells into effective and safe new modalities of care is still limited and further studies are needed before moving to patients with AKI.

Is-8: Cancer Immunotherapy with Genetically Modified T Cells

Bonini CH

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Adoptive T cell therapy is an innovative therapeutic approach aimed at providing effective and long-lasting tumor-reactive T cells to cancer patients. Unfortunately, T lymphocytes able to recognize tumor cells with sufficient avidity are often deleted or tolerized because tumor antigens are usually self-antigens. New technological gene transfer tools allow today to enforce natural T cells, enabling to generate high numbers of gene-modified tumor-reactive T cells from virtually every cancer patient. T cells have been manipulated ex vivo with viral vectors coding for tumor specific Receptors or "suicide" genes to potentiate their efficacy and minimize toxicity. The genetic transfer of a tumor-specific T cell receptor (TCR) in mature T cells has yielded promising clinical results in cancer patients. Nevertheless, the expression of a novel TCR into polyclonal T cells holds some limitations. In particular, the tumor-specific α and β TCR chains are expressed in lymphocytes that already bear an endogenous TCR on cell surface. Gene-modified cells thus express at least two different TCRs that compete for binding to the CD3 complex, and this bottleneck results in mutual TCR dilution and reduced avidity. Furthermore, since TCRs are heterodimers, the α and β chains of the endogenous and transgenic TCR can mispair to produce a new hybrid TCR, with unpredictable and potentially harmful specificity. To permanently remove the expression of the endogenous TCR and the risk of TCR chain mispairing, we developed a TCR gene editing approach, based on the combination of somatic knockout of the endogenous TCR genes (by transient expression of Zinc Finger Nucleases - ZFNs specific for the endogenous α and/or β TCR chains) and introduction of tumor-specific TCR genes by lentiviral vectors (Provasi, Genovese et al. Nat. Med. 2012). Challenges and opportunities of TCR gene transfer and TCR gene editing will be discussed.

Is-9: Memory T Cells with Stem-Cell Like Features in Health and Disease

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The ability to remember and respond more robustly in a second encounter with a pathogen is a critical property of the adaptive immune system and forms the basis of vaccination and adoptive cellular therapy of cancer. This process has been proposed to involve a stem cell-like memory T-cell subset, able to rapidly differentiate

in effectors and self-renew upon antigen re-encounter. The characterization of such a T-cell subset is not only of basic interest but also of clinical relevance for the development of strategies to target pathogens and cancer by adoptive T-cell therapy. Such a memory T-cell subset, referred to as memory stem T cells (T_{SCM}), has been recently described in humans (Gattinoni et al., Nat. Med. 2011). Our group identified conditions able to instruct naïve T cells into T_{SCM} cells *in vitro*, thus allowing their expansion and genetic modification in clinically compliant conditions (Cieri et al., Blood 2013). Gene-modified T_{SCM}, defined as postmitotic CD45RA+ CD62L+ CCR7+ IL-7Ra+ CD95+ T lymphocytes, are endowed with exceptional persistence and functional capacity *in vitro* and *in vivo*, which could be exploited for cancer adoptive immune-gene therapy. Nevertheless, while self-renewing T_{SCM} would be highly effective in providing long-term immune-surveillance against pathogens and cancer cells, this very same cell subpopulation may also represent a foe when considering T-cell mediated pathologies, such as autoimmune diseases and graft versus host disease (GvHD). In these clinically relevant contexts, T_{SCM} may represent a reservoir of long-lived T cells with undesired and detrimental specificities responsible for therapy resistance and high morbidity. Indeed, we have documented a selective accumulation of T_{SCM} early after allogeneic hematopoietic stem cell transplantation (HSCT), and our data on a small cohort of transplanted patients suggest that the extent of T_{SCM} accumulation one month after HSCT correlates with GvHD occurrence and severity. This presentation will discuss how T_{SCM} cells can be exploited in adoptive immunotherapy, and tamed in the context of T-cell mediated disorders.

Is-10: Design and Operation of A Multi-Product Cell Therapy GMP Manufacturing Facility in The USA

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The cell therapy manufacturing facility at the Center for Cell and Gene Therapy (CAGT) is one of the largest of its type at an American academic institution. It was designed to prepare a wide variety of cellular products for use in (i) early phase clinical trials and (ii) to support hematopoietic transplant programs at the affiliated adult and pediatric teaching hospitals. In addition, CAGT operates a contiguous viral vector manufacturing facility to prepare vectors for gene therapy studies. CAGT manufactures over 1,000 products and intermediates annually in support of 50 Investigational New Drug trials. This presentation will discuss and contrast the design and operation of these facilities and their support laboratories. Issues will include handling of multiple product types, environmental monitoring, compliance with USA FDA regulations, documentation, quality assurance and control programs, manufacturing for other institutions, flexibility to meet future demands and operational budgets. Progress towards transitioning cellular therapy products from unique cell preparations for individual patients to off-the-shelf generic products will also be presented

Is-11: Large Scale Production of Human Mesenchymal Stromal Cells for Use in The Treatment of Stroke

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Mesenchymal stromal cells (MSC) are under intensive investigation in regenerative medicine clinical trials and, because of their immunosuppressive properties, in studies to facilitate engraftment of hematopoietic cells and solid organs, and to prevent or treat graft-versus-host disease. The cells may be generated from bone marrow, adipose tissue and cord blood, and although their production is relative straightforward, it is labor intensive when using traditional flask-based culture techniques. To address this we have evaluated the Terumo Quantum bioreactor for the preparation of allogeneic MSC to be used for the treatment of stroke.
This is a hollow-fiber automated system which is seeded with 25ml whole marrow. The non-adherent cells are removed after ~24 hours and the adherent cells are fed via continuous flow of medium containing human platelet lysate. Growth is monitored by measuring glucose consumption and lactate production. The cells are harvested when the medium flow rate reaches 0.4ml/min and the lactate level is 4mM. The cells can then be seeded into a second bioreactor for additional expansion. MSC obtained using this method were essentially identical to those grown in tissue culture flasks with respect to immunophenotype, CFU-F production and tri-lineage differentiation. In a rat model of stroke they showed functional activity. The target MSC number of $2x10^8$ cells was obtained in half the number of passages and nine days earlier than when flasks were used for manufacturing. The number of open procedures was reduced from over 50,000 using flasks to 133 using the bioreactor. This manufacturing method was cleared by the US FDA for use in the stroke trial, which is currently accruing patients.

Is-12: Kidney Regeneration - Glomerular Perspective

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Objective: Podocyte loss is a major determinant of progressive chronic kidney disease. Although recent studies have shown that a subset of parietal epithelial cells can serve as podocyte progenitors, the role of podocyte regeneration in aging and nephron loss remains unclear.
Materials and Methods: Here we combined genetic fate mapping with highly efficient podocyte isolation protocols to precisely quantify podocyte turnover.
Results: We demonstrate for the first time that parietal epithelial cells can give rise to fully differentiated visceral epithelial cells indistinguishable from resident podocytes, and found that limited podocyte renewal occurs during glomerular development as well as in a diphtheria toxin-model of acute podocyte ablation. In contrast, the compensatory programs in response to nephron loss mainly evoke podocyte hypertrophy, but not podocyte regeneration. In addition, under physiological conditions no turnover of podocytes could be detected in aging mice. In the absence of podocyte replacement, progressive accumulation of oxidized proteins, deposits of protein aggregates, loss of podocytes and glomerulosclerosis were characteristic features of aging mouse kidneys.
Conclusion: In summary, quantitative investigation of podocyte regeneration *in vivo* provides novel insights into the mechanism and capacity of podocyte regeneration in mice. Our data reveal that podocyte generation is mainly confined to glomerular development and occurs after acute glomerular injury, but fails to regenerate podocytes in aging kidneys or in response to nephron loss.

Is-13: Kidney Regeneration – Tubular Perspective

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Objective: Acute kidney injury with tubular necrosis is a major complication of critical illness with high morbidity and mortality. To date it has not been clear whether tubular regeneration occurs out of a progenitor pool or whether resident tubular cells start proliferating.
Materials and Methods: Genetic fate mapping using several distinct promotors targeting different tubular cells combined with ischemia/ reperfusion (I/R) injury to precisely identify and quantify regeneration.
Results: Genetic labelling experiments were performed both with differentiated tubular cells and scattered tubular cells, which have been regarded as a tubular cell progenitor niche. Inducing I/R in both genetically labeled mouse lines revealed that proliferation after I/R injury only occurs out of the differentiated tubular cells. Several cellular markers were identified which characterize this dedifferentiation and proliferation process.
Conclusion: In summary, these cell fate tracking experiments of regeneration establish that regeneration of tubular cells after I/R occurs from any surviving tubular cell. Tubular cells appear to switch to a common injury response program characterized by the expression of a specific set of scattered tubular cell markers.

Is-14: The Augmented BMP Pluripotency Pathway via TGF-B Suppression Maintains The Ground State of Embryonic Stem Cells Self-Renewal

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Objective: Embryonic stem cells (ESCs) are pluripotent cells with capacity for differentiation into all cell types that are most frequently established from the inner cell mass (ICM) of blastocysts. However, the efficiency of ESC generation is influenced by genetic background in mice; as some strains are recalcitrance to mESC line derivation. Recently, we identified the suppression of mitogen-activated protein kinase (MAPK) kinase (also known as MEK) and transforming growth factor β (TGFβ) type I receptors by PD0325901 and SB431542, respectively-the combination named as Royan 2 inhibitors or R2i- enables the highly efficient derivation of pluripotent mouse embryonic stem cells (mESCs) from different strains. The cellular and molecular analysis indicated that R2i supports the ground state of pluripotency in a different route from well-known 2i condition which the latter inhibits MEK and glycogen synthase kinase 3 (GSK3) by PD0325901 and CHIR99021, respectively.
Materials and Methods: To investigate the molecular basis by which R2i maintains pluripotency, microarray analysis was performed in two mESC lines which were simultaneously cultivated for ten times in 2i and R2i. Our data revealed the significant elevation of BMP4-associated genes in R2i-grown cells in comparison to 2i-grown cells. For functional pathway analysis, BMP4 signaling was inhibited in R2i- and 2i-grown cells by adding noggin (500 nM) or noggin (250 nM) plus dorsomorphin (5 μM), two potent BMP signaling inhibitors.
Results: We observed no significant changes in the morphology of ES cells and Oct4 expression in 2i-grown cells in the presence of the BMP4 signaling inhibitors, even after several passages. However, the self-renewal capability of R2i-grown cells was strongly and adversely affected over a brief time period
Conclusion: Our analysis highlighted BMP signaling

as a pathway markedly induced by TGFβ inhibition in R2i-grown cells. Since several studies have indicated that BMP4 signaling through Smad1/5/8 suppresses developmental regulators such as neuroectodermal-associated genes and FGF signaling, we here demonstrated that R2i via activation of the 'differentiation-inhibiting' BMP4 signaling enhanced pluripotent state in mESCs even though this pathway seems to be dispensable in 2i culture condition.

Keywords: Embryonic Stem Cell, Pluripotency, Signaling Pathway, Small Molecule

Is-15: Neuronal Program Death Ligand 1/PD-L1 Central in Limiting Glioblastoma

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Objective: Activating the immune system to combat cancer has long been the focus of tumor immunologists. However the tumor microenvironment may impede immune responses, leading to ineffective or suboptimal tumor responses to immunotherapy. Therefore, understanding the immunosuppressive nature of the tumor microenvironment and stromal cells is essential for designing strategies that increase the likelihood of successful therapies and assist the tumor microenvironment in supporting beneficial immunity.

Glioblastoma multiforme (GBM) is a highly invasive tumor characterized by rapid growth, dismal prognoses, and resistance to standard treatments, yet GBM is relatively rare among primary cancers. We assume that brain-specific stromal cells are equipped to guard tissue serenity, and we postulate that common defects in immunologic genes (Ifnb and Pdl1) or the instability of stromal cells may be central to the development of chronic inflammation and cancer progression.

Materials and Methods: Surgical tissues from tumor mass and associated brain tissues were collected from patients with GBM. Immunoflorescent histochemistry was utilized to study differential expression of PD-L1 by neurons versus tumor cells. Experimental model of glioma was established in different gene modified mice. We utilize different blocking antibodies and or gene targeting techniques to knock out/overexpress genes or proteins in neurons to study their functions. We also utilized DNA microarray to identify differentially expressed genes in neurons.

Results: Lack of IFNβ led to the loss of program cell death ligand 1 (PD-L1) expression by the neurons. PD-L1 is a transmembrane protein and a negative regulator of T-cell activation via binding to known receptors (PD-1 and B7-1). Our investigation revealed that neuronal PD-L1 is instrumental in limiting glioma growth by inducing caspase-dependent cell death. Additionally we found out that neuronal PD-L1 expression is associated with better prognosis in patients with GBM.

Conclusion: It is likely that immunocompetent neurons (and other tissue-specific tumor stromal cells) sense and prevent tumor growth, partially through upregulation of IFNβ-dependent PD-L1, which binds to an unknown receptor on glioblastomas, thereby suppressing PD-L1 expression. Subsequently, this process results in tumor arrest by inducing caspase-dependent cell death. Neurons with immunogenetic defects, such as those lacking the Ifnb gene and its targets like pdl1, lack antitumor suppressive capacity. In this scenario, immunocompromised brain acts as a double-edged sword. It allows gliomagenesis and CNS inflammation, while it also tolerates tumor growth because neurons cannot engage immune receptors and hence are unable to induce tumor killing. The immune make up of the brain tissue versus tumors should be considered carefully to select suitable immunetherapy.

Is-16: Collagen Type I Modified Films for Regeneration of Corneal Endothelium

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Objective: Corneal transplantation, a common surgical protocol for visual acuity improvement, is limited owing to a shortage of high quality donor corneas and/or its accurate replication of structural and biochemical composition of native cornea in a scaffold. Construction of neo-corneas utilizing novel, biocompatible and biodegradable scaffold source, could address such formidable challenges. Herein, we designed optically transparent, micro-structurally stable poly(lactide-co-glycolide) (PLGA) and degummed silk films, surface-modified with collagen type I as an alternative scaffold source for bioengineering cornea.

Materials and Methods: First, silk from Bombyx mori and PLGA (Mw 90,000) were used to fabricate the transparent films, followed by its surface modified using collagen type I. The morphological and structural properties were analyzed by FE-SEM, AFM, FTIR, contact angle, etc. In vitro biological compatibility was studied such as morphology, initial attachment, proliferation, mRNA expression, and proteins related functions using primary corneal endothelial cells.

Results: FE-SEM images display the surface roughness, confirming the coating of collagen type I on the respective films. AFM results show collagen type I modified films have a higher Ra (nm) values than unmodified surfaces. Although, collagen type I and cultured cells did not demonstrate any alteration in transparency at visual wavelength, however, material type is able to produce some difference on transparency. Cultured corneal endothelial cells on collagen type I

modified surface showed better initial attachment, proliferation, and expression of mRNAs. Even though differences occur between collagen type I modified films, they do not show any significant differences on morphology, expression of ZO-1 and Na+/K+-ATPase and well-expressed their functional proteins which are regulated functions of corneal endothelium.

Conclusion: Overall, the results suggest that collagen type I modified surface of PLGA film and silk fibroin film may be a suitable alternative for high quality corneal tissue expansion and transplantation.

Is-17: Isolation and Characterization of Primary Human Bone Marrow Mesenchymal Stem Cells

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Objective: Human bone marrow (BM) contains a rare population of non-hematopoietic mesenchymal stem cells (MSC) that are of central importance for the hematopoietic microenvironment. We and others have shown that primary BM-MSC were enriched in lin-/CD45-/CD271+ cells but not in the CD271- cell fraction. Furthermore, we have shown that CD146 expression on primary BM-MSC allowed to discriminate endosteally- from perivascularly-located BM-MSC in human bone marrow in-situ (Tormin et al. Blood 2011, 117, 5067-5077). Although CFU-F frequencies in lin-/CD45-/CD271+ cells are as high as up to ca. 1 in 20, a more precise phenotypical definition of these rare cells is required to be able to study the exact cellular properties of this putative stem/progenitor cell population. We therefore aimed to identify novel and potentially better markers for the isolation and characterization of primary human MSC by utilizing comparative gene expression profiling on human lin-/CD45- BM cells sorted based on CD271 expression.

Materials and Methods: BM aspirates were from healthy volunteer donors. Primary MSC were pre-enriched by RosetteSep lineage depletion and then isolated by multi-color FACS sorting. Illumina gene arrays were used for comparative gene expression analysis. MSC properties were evaluated by standard *in vitro* and *in vivo* assays.

Results: In total, 219 genes were significantly upregulated in the CD271+ subset, including typical MSC genes as well as genes encoding for cytokines, growth factors and extracellular matrix protein. Twenty-eight of the upregulated genes related to surface-expressed molecules. Four of the genes were cell surface markers that had been previously used to isolate human MSC from different tissues, while the remaining 24 genes had not been reported in the context of MSC isolation.

FACS analysis of the expression of the potential novel markers on lin-/CD45-/CD271+ cells revealed two staining patterns, i.e. marker expression was either directly correlated with CD271 expression and did thus not enable to further enrich for CFU-F (e.g. CD151), or the marker was only expressed on a fraction of the lin-/CD45-/CD271+ cells, thus potentially allowing to identify a CFU-F and a non-CFU-F containing population within the CD271+ cells. In fact, sorting based on CD140a (PDGFR alpha) expression allowed to sort a population of lin-/CD45-/CD271+/CD140a$^{low/-}$ cells with a CFU-F frequency of 1 in 5. These cells furthermore demonstrated typical in vitro and in vivo stroma formation and differentiation capacities and exhibited high levels of genes associated with mesenchymal lineages as well as HSC supportive function. Moreover, lin-/CD45-/CD271+/CD140a$^{low/-}$ cells effectively mediated the *ex vivo* expansion of transplantable CD34+ hematopoietic stem cells.

Conclusion: Taken together, these data indicate that PDGFRα is a key marker for adult human BM MSC which enables to prospectively isolate a close to pure population of candidate stroma stem/progenitor cells. These results will enable to better characterize this important cellular component of the hematopoietic microenvironment.

Is-18: Role of Polycomb Repressors in Stem Cells, Cancer and Development

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Repressive Polycomb-group (Pc-G) protein complexes and the counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in the dynamic maintenance of proper gene expression patterns during development, acting at the level of chromatin structure. As such, they are important controllers of cell fate. When deregulated, these master switches of gene expression are strongly implicated in formation of a diverse set of cancers. An example is the Pc-G gene Bmi1 which is overexpressed in medulloblastoma, Non small cell lung cancer, hepatocellular carcinoma, prostate cancer, breast cancer and Glioma and is causally implicated in leukemia. We and others have recently implicated Bmi1/Pc-G as a critical regulator of stem cell fate in hemapoietic stem cells, neural stem cells, mammary epithelial precursor cells and ES cells. In addition, we have shown that Bmi1 is regulated by the Shh pathway and that the Ink4a/ARF tumors suppressors are

critical Bmi1 target genes in stem cells and in cancer formation. However our recent work on brain cancer (Glioma) and prostate cancer points to important ink4a/ARF-independent Bmi1 targets involved in adhesion and motility. Comprehensive profiling of Polycomb target genes in Drosophila revealed its crucial conserved role in repressing lineage differentiation pathways and morphogens, including Wg, Hh, Delta and Notch. Using genome wide *in vivo* 4C on larval brains we recently demonstrated that polycomb domains interact in 3D nuclear space and are guided by chromosome architecture. Furthermore, we have characterized in detail an essential E3-ubiquitin ligase activity in the PRC1 Polycomb complex that consists of a functional Ring1B-Bmi1 heterodimer. This E3 ligase activity is required for maintenance of Polycomb repression in normal- and cancer stem cells and hence offers potential novel ways to target cancer stem cells or tumor reforming cells in which the activity of this E3 ligase is hyperactivated. This is further substantiated by a novel way by which the activity of the Ring1B.Bmi1 E3 ligase is controlled which has also implications for a novel link between Polycomb silencing and control of Double-Strand DNA repair. The implications of these findings for stem cell biology, development, and possibilities for new translational approaches to cancer will be discussed.

Is-19: Unexpected Results of Prolonged Ezh2 Inhibition in An *In Vivo* Model for Glioblastoma

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Repressive Polycomb-group (Pc-G) protein complexes and the counteracting Trithorax-group (Trx-G) of nucleosome remodeling factors are involved in the dynamic maintenance of proper gene expression patterns during development, acting at the level of chromatin structure. As such, they are important controllers of cell fate and differentiation. When deregulated, these master switches of gene expression are strongly implicated in formation of a diverse set of cancers. Examples are the Pc-G gene Bmi1 and Ezh2 which are overexpressed in many cancers including Glioblastoma. However, recently Ezh2 has also been found to be mutated/inactivated in other forms of cancer, suggesting a highly context-dependent role as oncogene or tumor suppressor. An outstanding question is how to identify among the many Pc-G bound genes the cancer-relevant ones. hereto we have recently combined stringent ChIP-seq with custom shRNAi library screening in in vivo models for glioblastoma. As Ezh2 is associated with poor prognosis and metastasis in many cancer settings there is an increasing interest for development of selective Ezh2 inhibitors as potential new ways for epigenetic cancer therapy. We have developed conditional shRNAi inhibition in mouse models for aggressive Glioma to study the effects of Ezh2 inhibition on tumor initiation and maintenance. Whereas initial tumor regression upon Ezh2 inhibition was observed in vivo, prolonged Ezh2 inhibition caused unexpected profound changes in tumor plasticity, differentiation status with important consequences for tumor progression and treatment options, which will be discussed.

Is-20: Stromal Vascular Fraction Cells in Therapy of Degenerative Osteoarthritis: A Case-Control Clinical Study

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Is-21: Directed Differentiation and Programming of Human Pluripotent Stem Cells into Clinically Relevant Mesoderm

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Is-22: Mechanisms of Mesodermal Differentiation and Patterning in Pluripotent Mammalian Stem Cells

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Is-23: Development of The Vertebrate Musculo-Skeletal Axis

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The body axis of vertebrates is composed of a serial repetition of similar anatomical modules, termed segments or metameres. This particular mode of organization is especially conspicuous at the level of the periodic arrangement of vertebrae in the spine. The segmental pattern is established during embryogenesis when the

somites, the embryonic segments of vertebrates, are rhythmically produced from the paraxial mesoderm. This process involves the segmentation clock, a traveling oscillator that interacts with a maturation wave called the wavefront to produce the periodic series of somites. This clock drives the dynamic expression of cyclic genes in the presomitic mesoderm and requires Notch, FGF and Wnt signaling. Microarray studies of the mouse presomitic mesoderm transcriptome reveal that the segmentation clock drives the periodic expression of a large network of cyclic genes involved in cell signaling. In humans, mutations in the genes associated to the function of this oscillator result in abnormal segmentation of the vertebral column such as those seen in congenital scoliosis. Whereas the segmentation clock is thought to set the pace of vertebrate segmentation, the translation of this pulsation into the reiterated arrangement of segment boundaries along the AP axis involves dynamic gradients of FGF and Wnt signaling. The FGF signaling gradient is established based on an unusual mechanism involving mRNA decay which provides an efficient means to couple the spatio-temporal activation of segmentation to the posterior elongation of the embryo. Finally, the subsequent regional differentiation of the precursors of the vertebrae is controlled by *Hox* genes, whose collinear expression controls both gastrulation of somite precursors and their subsequent patterning into anatomical domains. Therefore somite development provides an outstanding paradigm to study patterning and differentiation in vertebrate embryos and a conceptual framework to explain human spine malformations, such as scoliosis.

Is-24: Making Muscle *In Vitro* from Embryonic Stem Cells

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Key cell types including skeletal muscle have proven difficult to differentiate *in vitro* from pluripotent cells. Differentiation of mature contractile muscle fibers *in vitro* from mouse or human pluripotent cells has so far not been reported. During embryonic development, skeletal muscles arise from somites, which derive from the presomitic mesoderm (PSM). Based on our understanding of PSM development, we established conditions allowing efficient differentiation of monolayer cultures of mouse embryonic stem (ES) cells into PSM-like cells without introduction of exogenous genetic material or cell sorting. To optimize the differentiation of ES cells toward the muscle lineage, we used a series of reporter ES cell lines, expressing fluorescent proteins under the control of genes specific for key stages of myogenic development. These reporter lines were used to sequentially optimize the differentiation conditions in order to reach maximal differentiation for each population. Our optimized conditions were inferred based on the development of the PSM *in vivo* and from a microarray series of early developmental stages of this tissue. We next established simple conditions to recapitulate primary and secondary/foetal myogenesis in vitro from these PSM-like cells. Our strategy allowed for the production of contractile fibers from pluripotent cells in vitro with an efficiency comparing well with current cardiomyocytes differentiation protocols. The muscle fibers produced are striated and multinucleated and exhibit post-natal characteristics. They also provide a niche allowing the development of Pax7-positive satellite-like cells. We used these conditions to differentiate ES cells derived from dystrophin-deficient *mdx* mice. We show that these fibers exhibit a strikingly abnormal organization of the myofibrils accompanied by a dramatic increase in the number of branches. While such a branched phenotype has been reported *in vivo* in *mdx* animals or in Duchenne patients, it has been attributed to fusion defects consequent to the cycles of regeneration occurring in dystrophic muscles. Our results rather argue that the defect is intrinsic to the fibers thus challenging current views on the origin of the pathology of Duchenne Muscular Dystrophy. Thus our work opens the possibility to study pathological mutations in mouse models for muscular dystrophies *in vitro*.

Is-25: Bioinspired Scaffold, Hydrogel and Stem Cell Based Strategies for Engineering Distinct Tissues

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Objective: The proper selection of a scaffold/matrix material is both a critical and a difficult choice that will determine the success or failure of any tissue engineering or regenerative medicine (TERM) strategy. In many cases a wide range of properties have to be carefully tuned for a specific application and to the use of specific cell populations.
We believe that natural origin polymeric biomaterials and bioinspired materials and processing routes and are the best choice for many distinct approaches. We have been working with this type of systems for the past 20 years, proposing a wide range of possibilities for the engineering of many different tissues. In addition, we have been publishing on an all range of innovative pro-

cessing methodologies to produce adequate scaffolds/ hydrogels for different TERM applications.
Furthermore an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation methodologies should be developed and optimized. In our research group we have developed many different strategies to deal with these issues.
Materials and Methods: We have been using different human cell sources namely: mesenchymal stem cells from bone marrow, mesenchymal stem cells from human adipose tissue, human cells from amniotic fluids and membranes and cells obtained from human umbilical cords. The potential of each type of cells, to be used to develop novel useful regeneration therapies has been studied. In many cases this involves the development of adequate co-culture methodologies. Cell uses and their interactions with different natural origin degradable scaffolds, hydrogels, and distinct nano and micro-carriers, and smart release/differentiation systems was assessed.
The studied materials include: starch based polymers, chitin, chitosan, gellan gum, casein, soy, algae based materials (ulvan, carragenan, algae derived ceramics), silk fibroin, gelatin, hyaluronic acid, marine collagen, among many others.
These different materials have been used to try to engineer different types of tissues, namely: bone, cartilage, osteochondral defects, intra-vertebral disk, skin, neurological tissues, tendon and meniscus
Results: In our research a great focus is given to the different sources of stem cells, the isolation of distinct sub-populations, ways of differentiating them, as well as their interactions with different 3D architectures and natural origin materials for culturing them. The use of bioreactors to control cell differentiation, as well as the surface modification of the materials in order to control cell adhesion and proliferation has also been studied in great detail. Several examples of biomimetic and nanotechnology based strategies to engineer an all range of distinct tissues will be described in the presentation.
Conclusion: Several 3D-architectures, novel hydrogel systems, biomimetic and nanotechnology based strategies to engineer different types of tissues will be described based on our many contributions to the literature in these fields. Examples for different combinations of natural materials and stem cells, and their application on the engineering of different tissues will be addressed.

Is-26: New Approaches for Tissue Engineering of Different Tissues Using Adult Stem Cells and Natural Origin Materials

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Is-27: Manufactured Oocytes and Sperm from Human Stem Cells: Just How Promiscuous are Cells *In Vitro*?

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The 2012 Nobel Prizes to John Gurdon and Shinya Yamanaka for their seminal work in nuclear transfer and induced pluripotency, coupled with the 2010 Nobel Prize to Bob Edwards for pioneering *in vitro* fertilization in humans, attests to the contemporary importance of stem cells and regenerative medicine for ART.
This lecture considers pluripotent stem cells, their differentiation into both oocytes and sperm *in vitro*, as well as their fidelity.
Our report entitled: Direct Differentiation of Human Pluripotent Stem Cells into Haploid Spermatogenic Cells notes that: *"Here, we show that hESCs and hiPSCs differentiate directly into advanced male germ cell lineages, including postmeiotic, spermatid-like cells, in vitro without genetic manipulation. These results demonstrate that male PSCs have the ability to differentiate directly into advanced germ cell lineages and may represent a novel strategy for studying spermatogenesis in vitro."* Understanding human gametogenesis is vital for improving infertility therapies and contraceptives. Patient-specific stem cells undergoing gametogenesis in vitro represent innovative models for mechanistic investigations and potential therapies.
Fertile offspring generated from oocytes derived from induced pluripotent stem cells. Hayashi et al. Report in Science. that: *"We show here that female embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) in mice are induced into primordial germ cell–like cells (PGCLCs), which, when aggregated with female gonadal somatic cells as reconstituted ovaries, undergo X-reactivation, imprint erasure, cyst formation, and exhibit meiotic potential. Upon transplantation ... contribute to fertile offspring."* This work teaches us surprising features about oogenesis in vitro IN MICE.
So unless your patients are rodents, please do not let 'fertile' imaginations run wild yet.
Cellular promiscuity: Explaining Cellular Fidelity *In Vivo* versus. Unrestrained Pluripotency In Vitro. The differentiation of pluripotent stem cells (PSCs) into various progeny, including now spermatids and oocytes, is perplexing.
This differentiation of pluripotent stem cells into various progeny, including now oocytes and spermatids, is perplexing. *In vivo*, Nature imposes strict fate constraints. Yet *in vitro*, reprogrammed PSCs liberated from the body government freely differentiate into any phenotype (except placenta), violating even somatic vs. germ cell segregations – raising concerns regarding 'Cellular Promiscuity.

Is-28: Epigenetic Regulation of Stem Cells during Reprogramming and Differentiation: Histone Deacetylases and Centrosomes

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Somatic Cell Nuclear Transfer (SCNT) and Induced pluripotency (iPS) in humans both provide unique patient-specific, disease-bearing pluripotent cell lines (PSCs) important to biomedical investigations, and yet the molecular mechanisms responsible for iPS are virtually unknown. Notwithstanding progress elucidating the roles of transcription factors and chromatin modifications during reprograming, cytoplasmic mediators remain largely unexplored. Here, evidence shows post-translationally modified microtubules (PTM), including mitotic spindle pole centrioles in human pluripotent stem cells (hPSCs), are involved in human iPS derivation. Acetylated a-tubulin antibodies targeting lysine 40 (Ac-Tub) detects subsets of assembled microtubules, including centrioles, in parent human foreskin fibroblast 1 (HFF1) cells. Following HFF1 reprogramming using four 'Yamanaka' factors, iPS cells show markedly increased centriolar Ac-Tub, unlike their progeny following in vitro differentiation. Antibodies to HDAC6, a cytoplasmic α-tubulin deacetylase (TDAC), strongly detect HFF1 mitotic centrosomes, but are lost following iPS reprogramming. Interestingly, HDAC6 centrosome labeling is restored after in vitro differentiation. Reduction of HFF1 HDAC6 levels with shRNA demonstrates strong microtubule hyperacetylation, including centrioles, and significantly reduces iPS colony formation. Conversely, HDAC6 over-expression reduces microtubule acetylation without impacting iPS reprogramming. Tubastatin-A (TubA), a chemical HDAC6 inhibitor, also hyperacetylates microtubules, with loss of centriole acetylation above 50 nM. Analogous to HDAC6 shRNA, TubA ≥ 100 nM significant blocks iPS colony formation. Collectively, the role of the cytoplasm and cytoskeleton in mediating reprogramming events, likely downstream from nuclear factors, demonstrates tight cooperation between nuclear dynamics and cytoskeletal plasticity. Perhaps microtubule PTM, like transcriptional regulation, is essential for iPS derivation, identifying an unappreciated example of cytoplasmic post-translational reprogramming. Supported by the US National Institutes of Health.

Is-29: The Global Regulatory Landscape for Cell-Based Products

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Is-30: Stem Cell Clinical Applications – Uses and Abuses: Ethics Issues

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Is-31: High-Performance ZnO Hollow Spheres Based Drug Carriers for Chemo-Photodynamic Cancer Therapy

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Objective: Development of novel, multimodal therapeutic nanocarriers for ultra-high medicine loading holds paramount promises in cancer diagnosis and therapy paradigm owing to the limitations of conventional chemotherapeutic drugs. Herein, we have developed photodynamic ZnO hollow spheres (ZHS) based therapeutic system for high performance intracellular delivery of anticancer drug (daunorubicin; DNR) in order to enhance drug efficacy per dosage in cancer therapy, followed by evaluation of drug-loading efficiency and synergetic chemo-photodynamic cytotoxic effects. Moreover, zinc oxide nanorods (ZNR) loaded with drug was also studied as a comparative material.
Materials and Methods: Low temperature solution grown ZHS was synthesized via two steps process; first, formation of ZnO-$Zn(OH)_2$ shell on the substrate surface; second, another layer of ZnO-$Zn(OH)_2$ is deposited on the step I resulted composite nanospheres surface followed by the formation of ZHS with addition of NH_4OH. The ZNR were also synthesized by aqueous solution method at low temperature, followed by their detailed characterizations. Further, daunorubicin drug was loaded into above materials under adequate stirring in dark conditions. The efficiency of ZHS-DNR and ZNR-DNR complex were studied by assessing their in vitro drug release, cellular internalization, DNR localization, cytotoxicity and apoptosis using CLSM microscopy, MTT assay, flow cytometry, etc.
Results: The ZHS-DNR complex comprises ultrahigh drug encapsulation efficiency (i.e. 87.7 ± 4.10%) compared to ZNR and previously reported photodynamic materials based drug cargos, which is ascribed to the high-specific surface area, hollow interior, nanostructured wall and good surface permeability. After cellular internalization into human alveolar adenocarcinoma

A549 cells, ZnO hollow spheres dissolves in acidic compartments of cancer cells, efficiently releases the loaded drug into lysosomes and successfully destruct cancer cell. Moreover, ZnO hollow spheres behave as a dual-purpose entity that not only serves as a drug carrier but also exhibit a synergetic photodynamic cytotoxic effect.

Conclusion: In view of ZnO structures therapeutic potential, the ZHS featured with hollow interior design and inherent photodynamic nature, have proved to be a powerful system as an efficient anticancer and therapeutics delivery agent.

Is-32: Processing of Peripheral Blood Progenitor Cell Products Using A Novel Acoustophoresis Platform

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Objective: Processing of peripheral blood progenitor cells (PBPC) for clinical transplantation or research applications aims to effectively select or deplete specific cell populations. Usually, fluorescence- or magnetically-based sorting techniques are used for PBPC processing. Here, we aimed to investigate whether a novel microchip-based ´acoustophoresis´ technique can be used for ultrasound separation of cell subsets from PBPC. In acoustophoresis, ultrasonic standing wave forces are applied to sort cells or particles in a continuously flowing suspension in a micro channel. Cells are separated from the surrounding media depending on their physical properties and sorting is primarily based on size, density and compressibility.

Materials and Methods: PBPC were obtained from patients and healthy donors undergoing PBPC apheresis. Cells were labeled with Dynal beads and sorted acoustically using a 2D-2Y chip and, in parallel, also magnetically. Sorted cells were analyzed by FACS and standard functional tests.

Results: We have previously reported that platelets can be effectively removed from PBPC samples with a depletion rate of about 90% in a label-free sorting system. Based on these data we now went on to investigate whether acoustophoresis could also be applied to realize sorting of defined leucocyte populations. PBPC samples from patients (n= 16) and healthy donors (n=6) were labelled with anti-CD4 microbeads (Dynal) and sorted on an acoustophoresis-microchip. In parallel, control magnetic sorting was performed. Mean purity of targeted $CD4^+$ cells was 87 ± 12% (± SD) for acoustophoresis, compared to 95 ± 7% for control magnetic sorting. Viability of sorted cells was very good for both sorting methods (95 ± 4% and 97 ± 3%, respectively). Acoustophoresis recovery rate of $CD4^+$ cells was 69 ± 19%, compared to 59 ± 19% for magnetic sorting. Furthermore, functional testing of targeted $CD4^+$ cells demonstrated unimpaired mitogen-mediated proliferation capacity and cytokine production.

Conclusion: In summary, the acoustophoresis technique can be utilized to efficiently sort bead-labelled lymphocyte populations from PBPC samples with high purity and recovery without impairing lymphocyte function. Acoustophoresis is, thus, an interesting technology for PBPC processing, which has furthermore the potential to offer a single platform technique for multi-parameter cell separation.

Ps-1: Evaluation of Expression of ZFX Gene in Human Astrocytoma Brain Tumors

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Objective: Astrocytomas are a type of cancer of the brain which are originated from astrocytes. Astrocytomas are the most common glioma. In recent years, stem-like tumor cells in glioma are detected. The zinc finger transcription factor, ZFX is an important regulator of self-renewal in multiple stem cell types. Moreover, ZFX expression abnormally increases in various cancers such as prostate adenocarcinoma, diffuse large B-cell lymphoma and follicular lymphoma.
Materials and Methods: In the current study, we used quantitative real time RT-PCR method for evaluation of ZFX expression in 25 human astrocytoma tumoral tissues with different grades. Fold changes in the expression of the target transcript were determined using the ΔΔCt method. Pvalues less than 0.05 were considered as statistically significant. Furthermore, the association of gene expression with various clinicopathological characteristics such as tumor grade, tumor size, gender, invasion, mitosis index, glomeroluid vessel formation, calcification and age were examined.
Results: We found that there is a significant association between ZFX gene expression and different tumor grades (p value = 0.008), the presence or absence of invasion (p value = 0.001), forming and non-forming of gelomeruloid vessels (p value = 0.002), the age over or under 50 years (p value = 0.001) and the presence or absence of calcification (p value = 0.001).
Conclusion: In overal, our results showed that there is a significant association between ZFX gene expression and various clinicopathological characteristics in astrocytomas. Our results implicate that ZFX contributes to the invasion of neoplastic cells, at least in part by neovasculogenesis. Due to this, ZFX may have the potential to be used as a target for therapeutic interventions in brain cancers.
Keywords: Astrocytoma, Tumor Grade, Gelomeruloid Vessels, Calcification

Ps-1: Evaluation of ZFX Expression in Meningioma

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Objective: The zinc finger transcription factor ZFX gene is situated on the mammalian X-chromosome. ZFX functions as an important regulator of self-renewal in various stem cell types such as embryonic and hematopoietic stem cells. That is very conserved between vertebrates. Furthermore, ZFX expression is abnormally elevated in several cancers, and positively correlates with tumor grade. Meningiomas are a diverse set of tumors arising from the meninges, the membrane layers surrounding the central nervous system. Meningiomas account for about 20% of all primary intracranial tumors. Recently, the presence of mesenchymal stem-like cells was proved in the meningiomas.
Materials and Methods: Due to this, for the first time, we examined the expression of ZFX using quantitative real time RT-PCR method in 25 meningioma tumoral tissues with different grades. Fold changes in the expression of the target transcript were determined using the Livak´s method. P-values less than 0.05 were considered as statistically significant. Furthermore, the association of gene expression with clinicopathological characteristics such as tumor grade and gender were examined.
Results: We found that there is a significant inverse association between ZFX gene expression and different tumor grades (p value = 0.028) and gender (p value = 0.009).
Conclusion: Therefore, it seems that ZFX may play a role in the initiation of meningioma and can be used as a therapeutic target in the future.
Keywords: ZFX, Meningioma, Real Time RT-PCR, Tumor Grade

Ps-3: Comparison of Immunophenotypic Characteristics and Proliferation Potential of Stem Cells Derived from Adult and Deciduous Teeth

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Objective: To understand whether stem cells derived from adult and deciduous teeth are closely related, we examined immunophenotypic pattern and proliferation potential of dental pulp stem cells (DPSCs) in comparison with cells isolated from human exfoliated deciduous teeth (SCHED).
Materials and Methods: Stem cells were isolated from retrieved teeth of donors who signed inform consent by Size-sieved Isolation technique. Expression pattern of hematopoietic (CD34, CD38, CD45 and CD133,), mesenchymal (CD29, CD44, D73, CD146, STRO-1 and CD105) and embryonic (SSEA-4 and OCT-4) stem cells markers was assessed in cultured DPSCs and SCHEDs using flow cytometric analysis. In order to examine cell proliferation, DPSCs and SCHEDs were cultivated for 1, 4, 7, and 10 days and cell proliferation rate was evaluated using the MTT assay.
Results: Hematopoietic stem cells markers exhibited a weak expression in both groups (≤2%). More than 98% of the cells in both groups were positive for Mesenchymal surface markers (CD29, CD44, and CD105). Expression of STRO-1, SSEA4, and OCT-4 were significantly higher in SCHED. Proliferation rate of DPSCs and SCHED showed no significant difference up to day 4, however significantly higher expansion rate of SCHEDs was shown compared to DPSCs at days 7^{th} and 10^{th}.
Conclusion: Both stem cells expressed common MSC markers. Permanent and deciduoustooth germs are derived from the ectoderm of the first branchial arch and the ectomesenchyme of the neural crest, however, different levels of expression of STRO-1, SSEA4, and OCT-4 suggests there might be more than one stem cell population existing within these tissues which differ in their embryonic status, and SCHEDs are a more primitive stem cell population in comparison to DPSCs.
Keywords: Immunophenotyping, Dental Pulp Stem Cell, Mesenchymal Stem Cells, Stem Cells of Human Exfoliated Deciduous Teeth

Ps-4: Potential of Chicken Feet Collagen as Scaffold for Tissue Engineering Applications

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Objective: Evaluation and characterization of chicken feet collagen and it's gel for *in vitro* proliferation of stem cells.
Materials and Methods: RPMI 1640 was purchased from Invitrogen included 10% (v/v) fetal bovine serum (FBS, Gibco) and 1,00 U penicillin streptomycin/ml were used as a medium. Hydrochloric acid (HCl 0.055 M) and pepsin (Sigma) 5% was used to extract the pepsin-solubilized collagen (PSC) from chicken feet for 40 hours. Derived collagen was characterized by using electrophoresis and the fourier transform infrared (FTIR) as well as X- ray diffraction (XRD) assay. Collagen gel was then prepared with 30 mg/ml concentration and tested for mechanical strength by Zwick/roell Z010. Fibroblast cells (Pasteur, Iran) were seeded at 1×10^6 cells/ml in collagen gel for study of cell behaviors.
Results: In electrophoresis pattern, three subunits (β, α1, α2) were observed, that was the same as bovine skin collagen. Indeed, band intensity of α1 and α2 chains were the same, suggesting collagen obtained from chicken feet has more than two types of collagens. Also, FTIR spectra demonstrated a similar pattern for collagens of two sources. XRD assay confirmed that there is hydroxyapatite component in the chicken feet collagen. Mechanical strengths of 31.2 and 3.3 KPa were observed for the bovine skin and chicken feet collagen gels, respectively. However it can be used for cell seeding and tissue engineering. Fibroblast cells could also proliferate well inside the chicken feet collagen gel with 10 times increase after 12 days.
Conclusion: Collagen extracted from chicken feet has potential to be used for tissue engineering applications as scaffold particularly for bone tissues due to the existence of hydroxyapatite in the extracted collagen.
Keywords: Chicken Feet Collagen, Scaffold, Collagen Gel, Characterization

Ps-5: The First Report about *In Vitro* Differentiation of Menstrual Blood Stem Cells into Keratinocyte-Like Cells

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Objective: Recently, some characteristic of menstrual blood stem cells (MenScs) such as non-invasive accessibility, refreshing and high proliferation capability have impelled scientists to take advantages of these cells for cell therapy of different diseases including skin disorders. In this study, we evaluated the transdifferentiation capability of MenSCs into keratinocyte.
Materials and Methods: The isolated stem cells from menstrual blood samples were characterized and subsequently co-cultured with seeded human keratinocytes on trans-wells up to 2 weeks. Thereafter, expression of keratinocyte markers such as Involucrin (IVL), Cytokeratin 14 (K14) and P63 was assessed in differ-

entiated cells in reference to undifferentiated cells using immunofluorescent staining and real-time PCR.
Results: Based on given analysis, the isolated MenSCs exhibited typical expression of keratinocyte markers at mRNA and protein levels. The immunocytochemistry staining showed that the differentiated MenSCs could express P63, K14 and IVL protein. Moreover, the expression levels of P63, K14 and IVL mRNA significantly up regulated in differentiated MenSCs compared to undifferentiated cells (6.59, 11, 2.94 fold, respectively, p=0.001 for all the three markers).
Conclusion: Our result demonstrated that MenSCs can develop to keratinocytes. The development of the method for efficient differentiation of MensScs into keratinocyte lineage will enable us to access the massive source of epithelial cells for treatment and healing purposes. But the future studies are required to found out applicability of these cells for clinical trial of skin diseases.
Keywords: Menstrual Blood Stem Cell, Keratinocyte, Differentiation

Ps-6: Mechanical Modulation Analysis of Mesenchymal Stem Cells under Pressure Loading

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Objective: Many mechanical factors affect stem cell behavior like pressure loading that may causes differentiation of a stem cell to chondrocyte. Mechanotransduction is a process by which many biochemical signals can be activated in cell components. Biomechanical pathways are known for this transfer and recent findings indicate that this loading causes cell deformation. In this study, using finite element method, mechanical behavior of stem cell components have been analyzed and specifically the magnitude of created strain in the cell under pressure has been studied.
Materials and Methods: Finite element methods have been used to study the pressure loading which is applied on a stem cell. The cell was placed between two plates and it was compressed by the upper one. Relationships between cell behaviour and the created deformation in the cell and its internal components such as the plasma membrane, nucleus and cytosol have been studied. To apply pressure deformation to the cell, FEM model was simulated using ADINA software (Adina RandD Inc., Watertown MA USA).
Results: Magnitudes of strain in the cell was reported by increasing displacement of the upper plate. The amount of strain was changed in nucleus but it was not as large as changes in cytosol. The value of created strain in cytosol was about 26.39% and in nucleus was about 0.63%.
Conclusion: In this study, the values of created strain in cell components under pressure loading are in agreement with strain values which have been measured by Delaine-Smith et al. for differentiation of stem cells and chondrogenesis in the laboratory (10% strain induced osteogenic genes 15% induced both osteo and chondrogenic genes) for differentiation of stem cells by pressure loading. Due to simplicity of our model as well as the intricate nature of cellular functions, although we may not be able to compare accurately the numerical results of this study with clinical reports of previous researches, but we can reveal order of significance of mechanical modulation effects of the applied mechanical stimulations on cellular activities through computational analysis.
Keywords: Mechanical Regulation of Gene Expression, Mechanical Properties, Finite Element Method, Cellular Mechanics

Ps-7: Effects of Indomethacin on Postnatal Development of Mouse Testes

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Objective: Indomethacin is a non-selective cyclooxygenase (COX) inhibitor that is commonly administered to extremely low gestational age neonates for symptomatic patent ductus arteriosus. It suppresses prostaglandins which modulate growth and secretion of various hormones. We examined the hypothesis that early postnatal administration of Indomethacin may influence growth and development of testes in neonatal mice.
Materials and Methods: The mice were acclimatized for seven days prior to coupling and were housed in an air conditioned animal house at 22 ± 2ºC with exposure to 10–12 hours of daylight. Day zero of pregnancy was determined by vaginal plug test. In this study, mice pups received intraperitoneally injection of 25, 50 and 100 mg/kg Indomethacin which was dissolved in 0.09% NaCl on the day of birth. After pup's delivery, male pups were sacrificed on day 35 and testes were removed, fixed in 10% neutral buffered formalin and embedded in paraffin wax. Serial sections (5µm) of the medullary area were obtained in each group and stained with hematoxylin-eosin. The sections were observed under light microscopy. For all experiments, at least 6 to 8 replicates were performed. The data were analyzed statistically using analysis of variance (ANOVA). A level of ($p<0.05$) was accepted as significant.
Results: Our results indicated that intraperitoneally injection of 25, 50 and 100mg/kg Indomethacin decreased the number of spermatogonia, spermatocyte and sperm in seminiferous tubule. The number of seminiferous tubules decreased in comparison to control ($p<0.05$).

Conclusion: These results provide evidence for the involvement of prostaglandins in the regulation of development and suppressing the growth of testis in the sucking mice. Therefore, early postnatal exposure to Indomethacin as a prostaglandins inhibitor may further exacerbate postnatal growth restriction of testis and ability to cope with stress.

Keywords: Indomethacin, Neonate,Testes, Mouse

Ps-8: Isolation, Culture and Characterization of Canine Mesenchymal Stem Cells

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Objective: Isolation and biological characterization of bone marrow derived mesenchymal stem cells (BM-MSCs) is the first step for cell therapy of different human and animal diseases. The objectives of the present study were isolation, culture and characterization of canine BM-MSCs.

Materials and Methods: Two clinically healthy large mixed breed dogs of 1 and 3-year-old were used. Bone marrow (20 ml) was collected from the proximal humerus under general anesthesia and cultured and passaged in αMEM and FBS. Cells of passages 4, 5, and 8 from older dog and passage 8 from younger one were seeded in 24-well plates at a density of approximately 5×10^4 cells per well, for 8 days and three wells per day were used to determine cell number. The population doubling time (PDT) at each passage was calculated. Karyotyping of cells of passages 1 and 8 were performed. The chromosome numbers were counted and evaluated to obtain the percentage of diploid cells. The mean and SE of counted cells in growth curve analysis were compared using independent sample t test and one way ANOVA (SPSS for Windows, version 11.5, $p\leq0.01$).

Results: BM-MSCs displayed fibroblastic-like morphology in all passages. The PDT of the passage 8 of younger dog was 41.1 hours and of the passages 4, 5, and 8 of older dog were 65.8, 55.3 and 90 hours, respectively. Proliferation of canine MSCs in younger donor was higher than adult donor. The chromosome number of the canine BM-MSCs was 2n=78 and the results showed that the BM-MSCs were normal.

Conclusion: The increase in the PDT of BM-MSCs occurs with the increase in age. Karyotyping of these cells was normal. These cells can be used for tissue repair and regeneration in most organs in medicine and veterinary medicine.

Keywords: Bone Marrow, Mesenchymal Stem Cell, Growth Curve, Karyotype, Dog

Ps-9: Hypoxia Preconditioning Improved Survival of Human Umbilical Cord Blood Derived Mesenchymal Stem Cells

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Objective: In the recent decade, human umbilical cord Blood derived mesenchymal stem cells (hUCB-MSCs) provide enormous potential for appropriate therapy in different fields. Researches have shown that hypoxia preconditioning (HP) of bone marrow derived Mesenchymal stem cells (BM -MSCS) enhance their survival and extend their life span and inhibit pro inflammatory chemokines in MSCS. In this study, we examined the proliferation capacity of hUCB-MSCs under hypoxic condition in comparison with normoxic status.

Materials and Methods: HUCB-MSCs were isolated. The Ethical approval was obtained from the institutional ethical review board at Blood transfusion Research Center. The isolated population have been characterized as mesenchymal stem cells by surface markers analysis and functional properties. UCB-MSCs were exposed to HP protocol consisted on 2 sets of hypoxia (15 minutes, 2.5% O_2) and re-oxygenation (30 minutes, 21% O_2), prior to hypoxic challenge (72 hours). Cell survival was measured by the trypan blue staining. Apoptosis was measured with MTT assay. Doubling time and doubling rate were calculated in hypoxic and normoxic groups.

Results: The results showed that HP status increases cell proliferation of UCB-MSCS (1.2 fold) in comparison with control group. The MTT results showed that cell viability of hUCB-MSCs after 72 hours of hypoxia preconditioning significantly increased (0.36 ± 0.07) in comparison with normoxic group (0.26 ± 0.07) ($p<0.05$).

Conclusion: Our results suggested that hypoxia preconditioning - UCB-MSCS could be a potential therapeutic avenue for increasing the efficacy of stem cell therapy and support future application of UCB-MSCS for regenerative medicine.

Keywords: Mesenchymal Stem Cells, Hypoxia Preconditioning, Cell Survival

Ps-10: Characteristics of CD146 Positive Portion of Pulp Polyp Derived Stem Cells

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Objective: Pulp polyps (chronic hyperplastic pulpitis) are pathological tissues produced in an inflammatory proliferative response within a tooth. We have previously isolated stem cells from them, the "Pulp Polyp Stem Cells (PPSCs)". PPSCs' cultures showed in heterogeneous phenotype with a low stem cell yielding rate. In the present study we evaluated the effect of CD146 isolation on the isolation of PPSCs.
Materials and Methods: Single cells from enzymatically digested pulp polyp tissues underwent CD146 isolation using magnetic cell sorting technique. The positive and negative portions were cultured and their morphological features, flowcytometric antigen panel and differentiation capacity was compared.
Results: Pulp polyp CD146 positive cells displayed spindle shape morphology with successful differentiation into adipocytes and osteoblasts. They also expressed key antigen markers such as STRO-1, CD73, CD90, and CD105. CD146 positive portion of the polyp derived cells formed an average of 90 ± 30.6 which was significantly higher than the negative counterpart with no colonies ($p = 0.014$)
Conclusion: The CD146 positive cells isolated from pulp polyps fulfill the criteria for Mesenchymal stem cell definition, while the negative portion could not. The results of this study showed that CD146 enrichment can more homogenized the cultures of PPSCs and the stem cell portion is restricted to positive portion of cells.
Keywords: Mesenchymal Stem Cell, Pulp Polyp, CD146

Ps-11: Myeloid Leukemia Cells Exhibit Higher Amounts of Telomerase Alternative Splicing Variants Compared to Hematopoietic Stem Cells

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Objective: Telomerase activity control has great importance in cancer research. There is a controversy about the role of alternative splicing of human telomerase (hTERT), the catalytic subunit of telomerase enzyme, in regulation of telomerase activity. We aimed to find any difference between hTERT Alternative Splicing Variants (ASV) as specific targets which may differ between normal hematopoietic cells and leukemia cells.
Materials and Methods: Hematopoietic Stem Cells (HSCs) were isolated using a Fluorescent activating cell sorting (FACS) technique. $CD34^{+}CD38^{-}$ cells were considered as long term HSCs. Acute myeloid leukemia cell line KG-1 were cultured and detached enzymatically. Quantitative amounts of four different ASV mRNAs of hTERT including full length, alpha deletion, beta deletion and alpha beta deletion was measured using Real Time PCR.
Results: Flowcytometric analysis showed successful isolation of HSCs. These cells expressed low amounts of hTERT mRNAs with equal frequency in all variants. KG-1 cancer cells showed large amounts of hTERT mRNAs with higher percentage of alpha variants.
Conclusion: In this study we have clearly showed that myeloid leukemia cells express higher amounts of telomerase ASV mRNAs compared to normal HSCs but there was no trend toward a specific variant. This can be one of the reasons of unlimited replicative potentials for these cells. However, alternative variants of this gene are not useful targets for differential therapy of cancerous cells without affecting normal HSCs.
Keywords: Telomerase, Alternative Splicing Variant, Cancer Cell Lines, Hematopoietic Stem Cell

Ps-12: Stem Cell Properties are Restricted to CD146 Positive Portion of Dental Pulp Derived Cultured Cells

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Objective: Routinely, cultures of dental pulp stem cells (DPSCs) are achieved by direct culturing of tissue derived single cell suspensions which are very heterogeneous and contaminated by fibroblasts. The purpose of this study is to examine that CD146 expression can discriminate between dental pulp fibroblasts (DPFs) and DPSCs.
Materials and Methods: Based on the CD146 expression, magnetic cell sorting was done to purify DPFs and DPSCs from cultures of pulp derived cells. Colony forming assay, differentiation, and flowcytometry was performed to characterize the cells.
Results: While every 10,000 of CD146 positive cells

formed 96 ± 18.7 colonies, CD146 negative portion could not form any colonies ($p<0.001$). In addition, only the CD146 positive cells could differentiate to adipocytes or osteoblasts. Staining and RT-PCR confirmed these results.

Conclusion: Our results reveal that only the CD146 positive portion of the dental pulp derived cells were able to form colonies and differentiate into osteoblasts or adipocytes and are thus true stem cells, while the CD146 negative cells failed to do so. CD146 expression can be considered as a discriminative factor between DPSCs and DPFs.

Keywords: Stem Cell, Fibroblast, Dental Pulp, CD146

Ps-13: Expression of Telomerase Alternative Splicing Variants Doesn't Change during Early Stages of Hematopoiesis

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Objective: Telomerase activity has great importance in maintenance of self renewality. There is a controversy about the role of alternative splicing of hTERT, the catalytic subunit of telomerase enzyme, in regulation of telomerase activity. We aimed to find any changes in hTERT alternative splicing variants during early stages of normal hematopoiesis as a regulator in diminishing the self renewality within more descendant progenies.

Materials and Methods: Hematopoietic stem cells (HSCs) were isolated using a fluorescent activating cell sorting (FACS) technique from bone marrow. $CD133^{+}CD34^{-}$ cells were considered as pluripotent stem cells, $CD34^{+}CD38^{-}$ cells as long term (LT)-HSCs and $CD34^{+}CD38^{-}$ cells as short term (ST)-HSCs. Quantitative amounts of four different Alternative splicing variant mRNAs of human telomerase (hTERT) including full length, alpha deletion, beta deletion and alpha beta deletion was measured using real time PCR.

Results: Flowcytometric analysis showed successful isolation of HSCs subsets. These cells expressed low amounts of hTERT mRNAs with equal frequency in all variants. There was only a slight elevation in the frequency of hTERT mRNA variants in the SH-HSCs.

Conclusion: In this study we recapitulated previous finding that HSCs show only slight amounts of telomerase mRNA with a step up from LT-HSCs to ST-HSCs. However, we could not find any specific alternative splicing variant mRNAs as a dominant variant and the pattern of expression of these variants didn't change during early stages of hematopoiesis. It can be conclude that alternative splicing does not have a significant role in regulation of telomerase activity in normal hematopoietic system.

Keywords: Telomerase, Alternative Splicing Variant (ASV), Hematopoietic Stem Cell, Hematopoiesis

Ps-14: Osteogenesis Induced by Nanofiber Scaffold in Bone Marrow Mesenchymal Stem Cells via Inhibition of Transforming Growth Factor-Beta Signaling Pathway

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Objective: One of the most promising ways to repair critical-size bone defects, is the fabrication of nanofiber scaffolds which are designed to mimic the structure of the natural bone tissue.

Materials and Methods: In this study, electrospinning technique was performed and poly L lactide nanofiber (PLLA) and nano hydroxyapatite coated- poly L lactide nanofiber (PLLA/nHA) were constructed. Then the effect of these scaffolds on the osteogenic differentiation of human bone marrow mesenchymal stem cells (BM-MSCs) was investigated *in vitro* under osteogenic induction in comparison with tissue culture plates (TCPs). Furthermore, Transforming growth factor beta1 (TGF- beta) signaling pathway activity has been analyzed as an important regulatory pathway of osteogenesis. For this purpose, TGF beta1 recombinant protein and SB431542 small molecule were used as inductive and inhibitory factors of this pathway respectively. After 7, 14 and 21 days, Alkaline phosphatase enzyme (ALP) activity and extracellular calcium deposition were measured.

Results: The results showed that cultured BM-MSCs on scaffolds, had higher ALP activity and calcium deposition in compare with TCPs substrates. Meanwhile PLLA/nHA scaffold was the most influential among the all groups. Furthermore, the inhibition of TGF beta signaling pathway by SB431542, led to the increase of ALP activity and calcium deposition and the induction of this pathway by TGF beta1 caused the decrease of ALP activity and calcium deposition.

Conclusion: These results suggest that scaffolds could influence intracellular signaling pathways by providing 3D substrate for cells, thus direct cell fate.

Keywords: Electrospinning, Nanofiber, Osteogenesis, TGF Beta1

Ps-15: The Role of Embryonic Sertoli

Cells in Maintenance of Spermatogonial Stem Cells

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Objective: Sertoli cells are unique somatic cells in testis and have close contact with spermatogonial stem cells (SSCs). Adult and embryonic Sertoli cells differ from significant ways. Structural and growth factors secretion changes occur in Sertoli cells as they mature. Our objective was to use embryonic Sertoli cells as a feeder layer for Spermatogonial stem cell maintenance.
Materials and Methods: Embryonic Sertoli cells were obtained from E14.5 testis. The isolated cells expressed vimentin in their cytoplasm. SSCs were isolated from adult male mice by enzymatic digestion and cultured on embryonic Sertoli cells for 1 and 2 weeks. After culturing, the expression level of MVH, ZBTB and C-kit were studied by real time-PCR. Then, SSCs were transplanted to azoospermic mice. After 2 months, the recipient testes were examined for spermatogenesis restoration.
Results: Our results showed that this co-culture system could increase the expression level of MVH and ZBTB, but decreases C-kit expression. After transplantation, the co-cultured SSCs could restore spermatogenesis to azoospermic mice model.
Conclusion: Our findings revealed that embryonic Sertoli cells could maintain SSCs in undifferentiated state *in vitro*. This co-culture model could improve the SSCs quality in long term culture and restoration of spermatogenesis *in vivo*.
Keywords: Embryonic Sertoli Cell, Proliferation, Spermatogonial Stem Cell, Transplantation

Ps-16: Very Small Embryonic-Like Stem Cells (VSELs): Characteristics, Functions and Regenerative Applications

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Objective: Very small embryonic-like stem cells (VSELs) are a group of recently-identified pluripotent stem cells (PSC) which are closely-related to the epiblast-derived migrating primordial germ cells-like cells. These stem cells (SCs) are age-dependent in content, and proved to play a crucial role in rejuvenation and longevity. In this study, we reviewed a number of research articles which have led to identification, isolation and clinical applications of these highly potential SCs.
Materials and Methods: There have been employed various molecular techniques for VSEL studies such as flow cytometric assay to sort VSELs from other bone marrow-derived (BM-derived) nucleated cells, cell culture for VSEL-DS formation, carrier chromatin-immunopercipitation (Carrier-ChIP) to evaluate the structure of oct4 promoter, bisulfite sequencing and combined bisulfite-restriction analysis (COBRA) to evaluate DNA methylation of promoters, RT-PCR, RQ-PCR, Immunocytochemistry and final statistical analysis.
Results: VSELs were isolated from immunofluorescent BM-derived nucleated cells by fluorescence-activated cell sorting, and measured in size using bead particles with standard diameters. Bisulfite sequencing results revealed the hypomethylated status and therefore transcriptionally active oct4 and nanog promoter in VSELs. Moreover, studies on the unique genomic imprinting patterns indicate a quiescent transcriptome of VSELs. RQ-PCR analysis shows a high level of expression for DNA methylation regulators like dnmts in VSELs. In addition, studies on VSEL-DSs indicate the gradual loss of pluripotentiality during the formation of VSEL-DSs.
Conclusion: In conclusion, molecular studies revealed that bone marrow harbors a population of oct4+ nanog+ SSEA1+ sca1+ lin- CD45- CXCR4+ SCs that serve as an age-dependent backup of PSCs in adult tissues for later-differentiation into monopotent tissue committed stem cells during clinical emergencies. Controlled by epigenetic reprogramming of parentally-imprinted genes, VSELs are regulated to resist to insulin/ insulin-like growth factor signaling pathway which maintains their normal quiescent status. However, in several emergency situations VSELs are activated and mobilized into peripheral blood contributing to tissue/ organ regeneration. Recently, VSELs are considered as potential SCs in regenerative medicine for clinical employment. Albeit controversial in their isolation strategies, VSELs are worth investigating to discover their potential role in longevity and rejuvenation.
Keywords: VSEL, CXCR4, Aging, Tissue Regeneration, Regenerative Medicine

Ps-17: Evaluation Emotional Changes -Mental Infertility Treatment and Presenting Strategy

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Objective: Infertility is defined as a couple not fertilized after one year of regular sexual intercourse without using contraception. More than half of infertile couples after unsuccessful fertility show some sorts of emotional mal-adjustments and social develops. The raw deal with their infertility issues are shock and de-

nial. Therefore, psychological factors, providing coping strategies and social supports lead to a reduction in inappropriate reactions of infertility. These psychological factors are the norm.

Materials and Methods: Studying an overview of the current internet search period 1989 to 2013 databases in iranian and international Scopus, ScienceDirect, PubMed, Cochran Library, ISI, magiran, Springer, Elsevier, SID with a collection of 110 essays that end, 56 articles with keywords: Infertility, Psychological factors, Psychological issues, Solutions were examined.

Results: The prevalence of infertility with 10-15% of the female, the male is unknown, each based on age, sex, education, occupation, state of income, length of marriage, type of infertility, duration of infertility, lifestyle, social protection emotional problems, mental health, depression, frustration, a sense of loss of value, loss of mental balance - stress, low self-esteem, resentment, stress, anxiety, fear, anger, shame, loneliness, jealousy, lack of social adjustment, feelings of rejection and isolation, threatening relations between couples, blame the other, not having a sense of control over their lives, feelings of incompetence and inaptitude is.

Conclusion: According to the results can be stated that in addition to physical therapy and assisted reproductive techniques to assess emotional - mental and we should pay special attention to counseling programs, because both are complement of each other. Equally that we want a high success rate in assisted reproductive technology have had psychological issues with planning and advice. also, group counseling, identification stages and the factors influencing personal decisions, manage and adaptation to stress, behavioral therapy, family therapy and family members' emotional attachment are examined.

Keywords: Infertility, Psychological Factors, Psychological Issues, Solutions, Evaluation

Ps-18: Investigation of Endothelial Cells Detachment under The Fluid Flow Shear Stress in A Parallel Plate Hollow Fiber Bioreactor

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Objective: One of the problems of the oxygenator device which is used during surgical procedures is thrombus formation on polymeric fibers after few hours of usage. Endothelialization of blood-contacting surfaces is one prominent solution for avoiding thrombus formation. However, developing a stable layer of endothelial cells under flow shear stress is an obstacle. In this research different ranges of shear stresses were computed using the computational fluid dynamics (CFD) procedure and then percentage of cell detachment under obtained fluid flow shears have been examined by using a simple parallel plate bioreactor.

Materials and Methods: COMSOL software was used for computing the shear stress applied on ECs by fluid flow. Human Umbilical Vein Endothelial Cells (HUVEC, NCBI Code: 554) were obtained from National Cell Bank of Iran. The surfaces of Silicon fiber with 0.6 mm ID (kindly donated by Raumedic, Germany) were modified with collagen type I solution. Different magnitudes of shear stresses were applied on cultured ECs on the outer surfaces of the hollow fiber in a bioreactor. The percent of dislodged cells from the fiber were measured by MTT assay.

Results: The experimental results demonstrated that by applying the shear stress of 0.03 and 0.09 dyn/cm^2 on cells none of them, shear stress of 0.17 dyn/cm^2, 6.6%, shear stress of 0.76 dyn/cm^2, 18% and shear stress of 2 dyn/cm^2, 33% of the cells were dislodged from the hollow fiber.

Conclusion: The results showed that this parallel plate bioreactor can help us to have a uniform shear stress on cells throughout the length of the fiber. For strengthening the cells attachment to the fiber outer surfaces, some improved surface modification methods have to be performed on the fiber. In this way, this approach can be used for oxygenators in the near future.

Keywords: Endothelial Cells, Shear Stress, Parallel Plate Bioreactor, Cell Detachment, MTT Assay

Ps-19: The Effect of Avocado/Soybean Unsaponifiables (Piascledine) on Viability of Human Articular Chondrocytes and Adipose Derived Stem Cells

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Objective: Avocado/soybean unsaponifiables (piascledine) are plant extracts that have been reported to stimulate secretion of extracellular matrix in osteoarticular diseases. So far, the effects of this composition on stem cells and differentiation have not been investigated. In this study the effect of piascledine on the viability of articular chondrocytes and adipose derived stem cells (ADSCs) cultured by micromass method were investigated to determine suitable dose for chondrogenesis of ADSCs and chondrocytes proliferation.

Materials and Methods: Articular chondrocytes and ADSCs were harvested in second passage (P2) and third passage (P3), respectively. In micromass culture,

aliquots of 2×10^5 cells in droplets (12.5 µl) were placed on each well in 24-well tissue culture plates. Different doses of piascledine include 1, 5 and 10 µg/ml prepared with both differentiation and nondifferentiation media. MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay was performed on days 4 and 7.

Results: The data indicated that concentrations of 1 µg/ml in both differentiation and nondifferentiation media on day 4, and 5 µg/ml in differentiation medium on day 7 are the suitable doses of piascledine for articular chondrocytes viability and proliferation. The best doses of this drug for viability and proliferation of ADSCs were obtained as 10 µg/ml in nondifferentiation medium on day 4, and 5 µg/ml in differentiation medium on days 4 and 7.

Conclusion: Piascledine treatment caused to promote viability and proliferation of both articular chondrocytes and ADSCs compared to controls. Also the 5 µg/ml concentration of this drug was suggested for future studies on cartilage extracellular matrix production by ADSCs.

Keywords: Piascledine, Adipose-Derived Stem Cells, Chondrocytes, Micromass Culture

Ps-20: Evaluation of Nanofiber Scaffold Poly (Є-caprolactone)/Gelatin Biocompatibility in Proliferation of Bone Marrow Mesenchymal Cells

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Objective: Tissue engineering is an interdisciplinary science that is based on the use of polymer scaffolds along with cells for the regeneration of defective tissues. Considering nano-meter size of the natural extracellular matrix components such as cavities and the diameter of their constitutive fibers, today's interest has been raised to utilizing nanofibrous scaffolds for tissue engineering. As well as the use of stem cells for tissue engineering has great importance due to the ability of these cells to differentiate into different cell types.

Materials and Methods: In this study, poly (Є-caprolactone)/gelatin nanofiber scaffolds were prepared and examined for growth and morphology of bone marrow derived mesenchymal stem cells on the scaffolds. In order to prepare nanofiber scaffold, poly (Є-caprolactone) and gelatin were mixed at a weight ratio of 70/30,dissolved in hexa flouro isopropanol (HFP) and electrospun.The bone marrow stem cells were seeded on nanofiber scaffolds and tissue culture plates (TCP) as control. MTT test was used to compare the growth and proliferation of cells on nanofiber scaffolds and TCP. The morphology of cells on nanofibers were also evaluated by SEM.

Results: Our results showed an average fiber diameter of 189 ± 56 nm for the nanofibrous scaffolds. The proliferation of cells on PCL/gel nanofibrous scaffolds was found to be higher than that on TCP, indicating that the PCL/gel scaffolds serve as a suitable substrate for cell proliferation. SEM results also showed cell spreading on the nanofibrous scaffolds which is consistent with MTT results.

Conclusion: The overall results of this study suggest suitability of PCL/gel nanofiber scaffold for growth and expansion of mesenchymal stem cells from bone marrow. Regarding to the expression of neurotrophic factors by these cells, nanofibrous scaffolds seeded with these cells can have a significant potential in the regeneration of damaged nerve tissue.

Keywords: Bone Marrow Mesenchymal Stem Cells, Proliferation, Nanofiber Scaffold

Ps-21: Biodegradation and Biocompatibility Evaluation of Mg-substituted Fluorapatite Coating Using SBF and Proliferation of Dental Pulp Mesenchymal Cells

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Objective: Many efforts have been made to improve the connection of implant material with hard tissue. Ability to establish a direct link between hydroxyapatite and bone hard tissue is due to the similarity of their chemical and biological structure. In our previous study construction and characterization of Mg-substituted fluorapatite coating was examined.

Materials and Methods: In the present study, in-vitro behaviour of Mg-substituted fluorapatite was evaluated. The biodegradation and bioactivity of prepared samples were evaluated by immersing the samples in the simulated body fluid (SBF) for various periods under physiological conditions. We also examined the growth rate and proliferation of dental pulp derived mesenchymal stem cells using on Mg-substituted fluorapatite coating via MTS test.

Results: According to our data, the pH value of SBF

containing the coated samples increased at the first day of experiment followed by a decreasing trend thereafter. The increase of the pH value observed at the first day was due to the dissolution of the coatings and the subsequent decline of pH was caused by sedimentation of apatite on the surface of the samples. By increasing of magnesium in the coatings, we found a reduction in PH of the solutions. This was due to further sedimentation of magnesium in the solutions, which indicated the merit of Mg-substituted fluorapatite than fluorapatite coating. Also, according to the MTS test results, a partial more proliferation rate was observed in Dental pulp cells cultured on the Mg-substituted fluorapatite coverage compared to conventional cell culture plates.

Conclusion: Overall, the results of this research indicated the suitability of Mg-substituted fluorapatite coating more than fluorapatite coating due to the greater deposition of apatite on the surface, as well as the appropriate growth and proliferation of dental pulp derived mesenchymal cells on the Mg-substituted fluorapatite coating.

Keywords: Mg-Substituted Fluorapatite, Simulated Body Fluid, Bioactivity, Dental Pulp Mesenchymal Cells, Biocompatibility

Ps-22: A Modified Stirred Tank Bioreactor for Self-Renewing Expansion of Hematopoietic Stem Cells

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Objective: Performance evaluation of a modified stirred tank bioreactor (MSTB) having low shear stress for Ex vivo expansion of hematopoietic cell line.

Materials and Methods: Medium was RPMI 1640 (Invitrogen/ 10% (v/v) fetal bovine serum (FBS, Gibco) with 1,00 U penicillin streptomycin/ml. U937 cell line (Invitrogen) as a model of hematopoietic stem cells (HSCs) was used to inoculate static and dynamic culture systems. To set up the MSTB system, cell proliferation place was separated from the stirred tank bioreactor (STB) and the system was under control by using a PLC system. Cell proliferation was evaluated by hemocytometer counting method. Cell morphology was also evaluated quantitatively by a parameter named cell roundness, Rc (Belview software). Cell surface marker of CD14 was also detected by flow cytometry (Becton-Dickinson) to test the cell self-renewal ability. Performance of the MSTB system was compared to the STB and T-flask cultures.

Results: The U937 cell showed about 97.5 ± 1.7, 80 ± 2.8 and 0.9 ± 1.3 fold for the MSTB and T-flasks and STB respectively. The almost all cells (93.7%) proliferated in the T-flasks during the 8-day culture period were $CD14^-$ cells. The percentage of $CD14^-$ cells, however, decreased to 73% and 11% in the cultures with MSTB and STB system at the same culture period. Interestingly, the frequencies of round-shaped cells (Rc > 0.9) for the T-flask, MSTB and STB cultures were 92.17% ± 2.1, 68% ± 4.9 and 18% ± 3.5 respectively. The MSTB system shows an acceptable performance as compared to the STB system due to providing lower shear stress on the cells.

Conclusion: A modified stirred tank bioreactor system showed an acceptable performance for self-renewing expansion of hematopoietic cell line as HSC-mimicking cells *in vitro*, showing potential of the MSTB to possible use in the expansion HSCs *in vitro*.

Keywords: Modified Stirred Tank Bioreactor, Hematopoietic Stem Cell, Shear Stress, Self-Renewing Expansion

Ps-23: A Modified Stirred Tank Bioreactor for Expansion of Mock Hematopoietic Stem Cells

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Objective: Evaluate a performance of Modified Stirred Tank Bioreactor (MSTB) with low shear stress and simple appliance for ex vivo Expansion of Hematopoietic Stem Cells using hematopoietic cell line (U937).

Materials and Methods: RPMI 1640 was purchased from Invitrogen included 10% (v/v) fetal bovine serum (FBS, Gibco), 1,00 U penicillin streptomycin/ml were used as a medium. U937 cell line (Invitrogen) as mock HSCs were used to inoculate static and dynamic culture systems. Modified stirred tank bioreactor (MSTB) System fabricated in Sahand University of Technology was used as dynamic system. Cell surface marker CD14 antigen was detected using reagents. The treated and/ or untreated cells were incubated simultaneously with fluorescein isothiocyanate (FITC)-conjugated CD14 monoclonal antibody (Invitrogen). After washing, at least 104 cells were analyzed by flow cytometry (Becton-Dickinson, NJ, USA).The morphology of cells was also evaluated quantitatively by a parameter named cell roundness, Rc as described elsewhere. The cell roundness, Rc=2(Ac) 0.5/lc, was determined with area, Ac, and peripheral length, lc, of each cell by image processing software (Belview).

Results: The U937 cell as mock HSCs showed about 97.5 ± 1.7 and 80 ± 2.8-fold for MSTB and T-flasks respectively. The almost all cells proliferated in the T-flasks during the 8-day culture period were CD14- cells 93.7%. The percentage of CD14- cells, however, de-

creased to 73% in the cultures with MSTB system at the same culture period. The frequencies of round-shaped cells (Rc > 0.9) for the T-flask and MSTB cultures were 92.17% ± 2.1 and 68% ± 4.9 respectively. The MSTB system shows an acceptable performance in this regard due to providing lower shear stress on the cells.

Conclusion: In this work, a modified stirred tank bioreactor system was introduced for possible use in HSCs expansion *in vitro* with reducing shear stress exerted on the cells. U937 cells showed the considerable increase in the cell proliferation when they were cultured in the modified bioreactor, as compared to the cultures in T-flask. Majority of the cells cultured in the T-flask and MSTB were the round-shaped and $CD14^-$ cells after 8 days. The present study showed the potential of the MSTB to use in the expansion of suspension culture of stem cells *in vitro* such as HSCs.

Keywords: Modified Stirred Tank Bioreactor, Hematopoietic Stem Cell, Shear Stress

Ps-24: The Efficacy of Nanocarrier-Loaded Cotrimoxazole in Treatment of Brucella's Infection

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Objective: Intracellular bacterial infections are very important because the immune system and antibiotics used for eradication of these bacteria have limited access to these bacteria. In many cases high doses of these antibiotics are used in order to increase their efficacy but other commensal bacteria are affected either; and thus side effects like gastric problems will appear.

Materials and Methods: Loading of Cotrimoxazle in nanocarrier, investigating the toxicity of nanocarrier-loaded Cotrimoxazle on J774 (murine phagocyte) via MTT assay and examining the efficacy of Cotrimoxazole on intracellular brucella-infected murine phagocytes through CFU method.

Results: standard curve for Cotrimoxazole y= 0.0675x+0.0699R^2= 0.9929, MTT result for Cotrimoxazole: LD50 for Nanocarrier~217mM LD50 for Cotrimoxazole~1954.20mM LD50 for Cotrimoxazole in nanocarrier~196.66mM, brucella reducing number in response to loaded-Cotrimoxazole

Conclusion: Increasing the efficacy of Cotrimoxazole in eradicating Brucella (through augmenting their capacity to penetrate in to cells) and reducing consuming dose.

Keywords: Colony-Forming Unit (CFU), Intracellular Bacteria, Mouse Phagocytic Cells J774

Ps-25: The Impact of Platelet Factor Rich Supernatant as An Effective Alternative Source to Fetal Bovine Serum for Umbilical Cord Blood Mesenchymal Stem Cells Proliferation

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Objective: Umbilical Cord Blood derived Mesenchymal Stem Cells (UCB-MSCs) are promising candidates for cell-based therapy. Fetal Bovine Serum (FBS) is commonly used as a cell culture medium additive for *in vitro* UCB-MSCs expansion. However, the use of animal sera for human cell-based culture may be associated with ethical, and safety issues. Among the possible alternatives to the animal serum, platelet derived compounds have been proposed.

Materials and Methods: For preparation of PFRS, whole-blood donation of four healthy donors was used to prepare one pooled platelet concentrate out of buffy coats. Then, platelet concentrate was activated by human thrombin and calcium chloride. UCB-MSCs were obtained with informed consent and cultured in Dulbecco's modified Eagle's medium-low Glucose (DMEM), supplemented with 10% FBS and characterized with flow-cytometry and differentiated into the adipogenic and osteogenic lineages. Then for comparison, UCB-MSCs passages 4-8 were cultured in expansion medium consisted of DMEM containing either 10% FBS as a control group or 10% PFRS as a test group; then cell viability and population doubling rate for two groups were performed. To assess cell viability, 5000 cells were plated in each well of a 24-well plate and cell viability was then evaluated at different time points (T24h, T48h, T72h, T96h, 120h) via MTT. To determine the cell proliferation and population doubling rate at each passages, 2000 cells per square centimeter were plated in T25 flasks then UCB-MSCs were counted and passaged at a confluence of 80%.

Results: MTT results did not show significant increase in cell viability after adding PFRS to the culture medium in compare to FBS. Cell counting in different passages exhibited significant increase in population doubling rate (3.7 ± 0.36 vs. 3 ± 0.4, $p \leq 0.05$), and fold expansion (1.6 fold) in culture media containing PFRS.

Conclusion: Our results demonstrated the PFRS application as a safe, rich and ethically approved source that can be substituted with FBS in cellular therapy. In addition, functional capability of PFRS would be approved in animal model as well.

Keywords: Umbilical Cord Blood, Mesenchymal Stem Cells, Platelet Factor Rich Supernatant

Ps-26: Wharton's Jelly-Derived Mesenchymal Stem Cells Can

Differentiate into Hepatocyte-Like Cells by Hepatocyte Extract

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Objective: Wharton's jelly is an unlimited source of stem cells that can be used in cell therapy and tissue engineering without any ethical concern. It has been revealed the cell-free extract could be effective to induce cell differentiation. The objective of this study was to induce Wharton's jelly-derived mesenchymal stem cells (MSCs) into hepatocyte-like cells by premeabilization of the cells in the presence of hepatocyte extract.
Materials and Methods: MSCs were isolated from the umbilical cord,CD marker profile and their differentiation potential into adipogenic and osteogenic lineages were determined. The cells were then, permeabilized by streptolysin O in the presence of hepatocyte extract. The treated cells were cultured for 17 days. The cell phenotype was evaluated and the hepatocyte specific markers were detected by immunoflurecence and immunohistochemistry. The periodic acid Schiff (PAS) reaction and the cellular uptake of indocyanine green were performed to evaluate the functional behavior of the differentiated cells.
Results: The phenotype of extract-treated MSCs changed into round or polygonal cells with few short processes and they could express high levels of albumin, cytokeratin 18 and 19. The MSCs also could store glycogen and uptake and release indocyanine green.
Conclusion: We demonstrated for the first time that Wharton's jelly-derived MSCs could differentiate into hepatocyte-like cells by premeabilization of them in the presence of hepatocyte extract. This study suggests a feasible method to differentiate MSCs into functional hepatocyte-like cells.
Keywords: Wharton's Jelly, Mesenchymal Stem Cells, Differentiation, Hepatocyte Extract

Ps-27: Construction of A Recombinant Eukaryotic Expression Vector Containing Human Toll-Like Receptor 4 (TLR4) and MD2 Genes and Its Expression in HEK Cells, as A Whole-Cellular Reporter to Monitor The Inflammatory Condition of Cells

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Objective: Toll-like receptor 4 (TLR4) is a transmembrane pattern-recognition receptor (PRR) that initiates signals in response to diverse pathogen-associated molecular patterns (PAMPs), especially LPS. The key roles of toll-like receptor 4 (TLR4) as a mediator of the detection and responses of immune cells to invading pathogens are well known. Until recently, TLRs have been examined predominantly for their contribution to immune-related disorders. However, cumulative evidence suggests that TLR4 not only contributes to pathophysiology, but also plays a vital role in facilitating neurodegenerative conditions. Recently, there has been an increasing number of studies about the role of TLRs in the pathogenesis of brain disorders such as ischemic stroke, Alzheimer's disease and multiple sclerosis, as well as the therapeutic potential of TLR intervention in such diseases. However, most of the evidence for the role of TLR4 is observational and little is known of the molecular mechanism. In the current study, to assess the molecular mechanisms of inflammation in a cell reporter moed we have constructed a recombinant eukaryotic vector expressing the TLR4 and MD2 encoding sequences. By implementing aforementioned vector we have established a stably transformed human embryonic kidney (HEK) cell line for studying the molecular events behind the inflammation.
Materials and Methods: Using genetic engineering approaches, we successfully constructed a recombinant pBudCE4.1 (+) containing CDS of TLR4 and MD2. This vector was transfected into the HEK cells. The stable transformants were isolated by addition of pouromycin. Resistant cells were isolated for further experiments.
Results: Expression of MD2 and TLR4 was confirmed in isolated stable cells. Moreover the functional activity assessment using LPS indicated increased expression of TNFα, iNOS and TLR4 in these cells. These results indicate that transfection of expression vector encoding human TLR4 and MD2 into the HEK cells is sufficient for the activation of the TLR4 by LPS.
Conclusion: We have established a stable cell line appropriate for study of inflammation. This system is approved to be feasible for future studies especially as a cell model to chase the inflammation processes.
Keywords: TLR4, Toll-Like Receptor 4

Ps-28: Effects of Steroid Hormones on The Expression Pattern of Plueripotency Markers in The Mice Uterine Tissue

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Objective: It has been suggested that endometrial stem cells are likely responsible for endometrial regeneration. The aim of this study was to investigate the effect of estradiol and progesterone hormones on the Oct4 and Sox2 mRNA expression of uterine tissue in the ovariectomized mice.
Materials and Methods: Adult female NMRI mice were underwent ovariectomy. Two weeks later, the mice were treated with 17β-estradiol, progesterone and combination of estradiol and progesterone for 5 days and then were sacrificed and their uterus removed. Quantitative Real time PCR of pluripotency markers (Oct4 and Sox2) was performed.
Results: Comparison of the normalized cycle of threshold (C_T) values revealed that levels of Oct4 gene expression in the estradiol - progesterone combined treatment group in comparision to other groups was significantly decreased ($p<0.05$). Whereas There were no significant differences between Oct4 gene expression in the control, estradiol, Progesterone groups ($p>0.05$). Levels of Sox2 gene expression in the 17β-estradiol treated and estradiol - progesterone combined treatment groups were significantly decreased in comparision to other groups ($p<0.05$).
Conclusion: The present study indicated that expression of the Oct4 and Sox2 mRNA in the uterine tissue were affected by steroid hormones.
Keywords: Steroid Hormones, Uterine, Pluripotency Markers

Ps-29: A Heparin Binding Growth Factor Concentration in Association with Inflammatory Cytokines in Multiple Sclerosis Patients

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Objective: A heparin-binding growth factor known as Midkine (MK) possess various effects in different tissues of the body, including an important role in induction of oncogenesis, inflammation and restoration of tissues. MK with promoting effects in inflammatory responses through enhancing the leukocytes migration in multiple sclerosis (MS) patients. The present study aimed to assess the concentration of this growth factor which correlated to inflammatory cytokines.
Materials and Methods: To evaluate the MK levels and its relationship with inflammatory cytokines (IL-17 and IL-23) in MS patients, the serum concentration of these proteins was assessed by ELISA in 32 MS patients in comparison with 32 healthy subjects.
Results: Our results showed that the MK concentration in MS patients is lower than healthy controls. Also we observed a significant decrease in IL-23 cytokine levels in MS patients.
Conclusion: There was a significant correlation between MK and IL23 concentrations in our study cohort. These results confirms a role for MK in inflammatory reactions in MS.
Keywords: Midkine, IL17, IL23, Multiple Sclerosis

Ps-30: Assessment of Circulating Endothelial Progenitor Cells in Multiple Sclerosis Patients with Optic Neuritis Attack

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Objective: Optic neuritis (ON) is inflammation of the optic nerve. In multiple sclerosis (MS) acute demyelization of optic nerve is a common cause of ON in some parts. Endothelial progenitor cells (EPCs) are present in circulation and contribute to vasculogenesis in adults. The aim of present study was to determine the number of circulating EPCs in MS patients with ON.
Materials and Methods: Blood samples were collected from 50 MS patients with ON and 40 healthy individuals (mean ages: 26 ± 5). Together with the collection of demographic data, a sample of venous blood was drawn to assess a complete blood count with differential; circulating, VEGF2, CD31, CD144 and CD309 as ECs markers and CD34 as hematopoietic cell marker using flow cytometry. Data are expressed as mean ± SD. Results were analyzed statistically, using the Independent sample t test. Values of $p<0.001$ were taken as significant.
Results: The median absolute percentage of circulating CD34+ HPCs in the overall population of ON patients was 18 ± 9.96. Against in the healthy was 26.31 ± 14.33 % ($p<0.001$). Patients with ON had EPCs which it was significantly higher than in healthy controls. In addition, the large number of EPCs but less number of circulating CD34 hematopoietic progenitor cells detected in patients.
Conclusion: Overall, data indicated EPCs circulate in peripheral blood of MS patients with an early phase of ON. It seems to increase of EPC relates to a good outcome. It may be that Heightened mobilization of EPCs represents an important role in prevention from BBB disruption or may be caused neoangiogenesis in MS patients with ON.
Keywords: Multiple Sclerosis, Endothelial Progenitor

Cells, Optic Neuritis

Ps-31: Presence of Dental Mesenchymal Stem Cells in Oral Reactive Lesions by Immunofluorecence Method

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Objective: Stem cells are innovative tools for tissue regeneration. Several niches in adult human body are colonized by multipotent stem cells but access to these potential reservoirs is often a limiting point. Although human dental pulp stem cells isolated from healthy teeth have been extensively characterized, it is unknown whether stem cells also exist in the reactive lesions of oral cavity such as pyogenic granuloma and peripheral ossifying fibroma which are kind of inflammatory proliferation of different cell families. Here we explored whether cells retrieved from such lesions have dental mesenchymal stem cell (DMSCs) or not.
Materials and Methods: Two pyogenic granulomas and two peripheral ossifying fibromas were achieved through excisional biopsy and preserved in PBS-EDTA in -86°C. Then we cut them in 5µm diameter with Cryostat. After washing these samples with PBS we used anti-STRO-1 primary antibody that is a monoclonal IgM derived from mice; after that we applied secondary antibody that was goat anti-mouse IgM-FITC to detect STRO-1+ cells by immunoflourescence technique.
Results: Immunofluorscence microscopy revealed presence of STRO-1+ cells in these lesions, particularly localized at perivascular zone. The negative control group wasn't glowing.
Conclusion: Based on these results, it was found that reactive lesions of pyogenic granuloma and peripheral ossifying fibroma have STRO-1 positive mesenchymal cells, which raises the possibility that these cells can be used as a new, accessible and non-invasive source of stem cells.
Keywords: Pyogenic Granuloma, Peripheral Ossifying Fibroma, Immunoflourescence Technique, Dental Mesenchymal Stem Cell, STRO-1

Ps-32: The Effects of Poly I-C Treated Mouse Bone Marrow-Derived Mesenchymal Stromal Cells and Its Supernatant on NK Cells Activity against Yac-1 Cell Line

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Objective: Mesenchymal stromal cells (MSCs) are multipotent, non-hematopoietic precursor cells that could be found in many adult textures. Multipotency and immunomedulatory potential of MSCs makes these cells as a remarkable tools for treatment of some diseases. It seems that stimulation of toll like receptors expressed on the surface of mesenchymal stromal cells may be potentiated immunomedulatory effects of these cells. This study was done to investigate the effects of polarized bone marrow-derived mesenchymal stromal cells of mouse and its supernatant on cytotoxic activity of natural killer (NK) cells.
Materials and Methods: MSCs isolated from bone marrow of femur and tibia of NMRI-mice. Third passage of cells were treated with Poly:IC. Then the effects of polarized MSCs and its supernatant evaluated on cytotoxic activity of NK Cells on lymphoid cancer cells Yac-1 (as target cells) using flow cytometery.
Results: MSCs treated with poly I-C and its supernatant made the survival rate of Yac-1cells increased and the percent of dead cells (apoptosis and necrosis) decreased in comparison to untreated MSCs.
Conclusion: MSCs treated with poly I-C and its supernatant inhibited the cytotoxic effects of NK cells on Yac-1 cell line.
Keywords: Mesenchymal Stromal Cells, Natural Killer, Poly I-C

Ps-33: Evaluation of LPS Treated Mouse Bone Marrow-Derived Mesenchymal Stromal Cells and Its Supernatant on NK Cells Activity Against Yac-1 Cell Line

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Objective: Mesenchymal stromal cells (MSCs) are multipotent, non-hematopoietic precursor cells that could be found in many adult textures. Multipotency and immunomedulatory potential of MSCs makes these cells as a remarkable tools for treatment of some diseases. It seems that stimulation of toll like receptors expressed on the surface of mesenchymal stromal cells may be potentiated immunomedulatory potential of these cells. This study was done to investigate the ef-

fects of polarized bone marrow-derived mesenchymal stromal cells of mouse and its supernatant on the cytotoxic activity of natural killer (NK) cells.
Materials and Methods: MSCs were isolated from bone marrow of femur and tibia of NMRI-mice. Third passage of cells were treated with LPS. Then the effects of polarized MSCs and its supernatant evaluated on cytotoxic activity of NK Cells on lymphoid cancer cells Yac-1 (as target cells) using flow cytometery .
Results: MSCs treated with LPS and its supernatant made the survival rate of Yac-1cells decreased and the percent of dead cells (apoptosis and necrosis) increased in comparison to untreated MSCs.
Conclusion: This results indicated that MSCs treated with LPS and its supernatant decreased the inhibitory effects of MSCs on NK cells.
Keywords: Mesenchymal Stromal Cells (MSCs), Natural Kiler (NK), LPS

Ps-34: Effect of Crocin on Differentiation of Neural Stem Cells to Oligodendrocytes

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Objective: Oligodendrocytes are myelin forming cells that develop from proliferating Oligodendrocytes precursor cells (OPCs), and damaged in multiple sclerosis (MS) disease. Recent studies focus on neuroprotective factors that promote neural stem cells differentiation to Oligodendrocytes for the aim of cell therapy in MS patients. Crocin is the main carotenoid of saffron that has neuroprotective effects. In current study, we evaluated how it might differentiate neural stem cells to oligodendrocytes.
Materials and Methods: In this study, neural stem cells were isolated from embryos cortex of E14 pregnant rat. Optimum concentration of crocin extract was determined using alamar blue. Neural stem Cells (NSCs) were divided into 3 groups and treated with Media (DMEM- F12): The first group treated with bFGF (10ng/ml), the second group : bFGF(10ng/ml) and PDGF-AA(30 ng/ml) and the third group: 100 mM of crocin solution for 24 hours, 4 and 6 days. The expression of OLIG2 marker as early OPCs marker was assayed by flowcytometry technique. The level of SOX10 (a transcription factor in differentiation of neural cells) mRNA was evaluated by RT-PCR. MBP (myelin basic protein) expression was compared between different groups in the late stages of differentiation. The positive control for MBP was PDGF-AA (30 ng/ml) + laminin (1 ng/ml).
Results: Flowcytometrys data showed the expression of olig2 in crocin group (96%) is similar to positive control group (98%). According to RT-PCR results the expression of sox10 in two above groups was the same.
Conclusion: Our data demonstrates that crocin has potent role on olig2 and sox10 expression, but not effective improvement on MBP marker. Therefore, crocin could stimulate early neural stem cells differentiation to OPCS (oligodendrocyte precursor cells).
Keywords: Differentiation, Neural Stem Cells, Crocin, Oligodendrocytes

Ps-35: Dehydroepiandrosterone Treatment Can Increase GSK3β Phosphorylation in Neural Progenitor Cells

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Objective: Alzheimer disease (AD) is the most common form of dementia. There is no treatment for this disease, eventually leads to death. In the early stages, the most common symptom is difficulty in remembering recent events. Research indicates that some genetics factors and fracture of special protein in brain produce and deposit aging plaques called amyloid bodies. Studies have shown that any disruption in wnt signaling pathway is associated with AD. One of the important molecules involved in activation or inactivation of this pathway is glycogen syntase kinase3β (GSK3β). The main goal of this study was evaluation of GSK3β phosphorylation by treatment of cells with dihydroepiandrosterone (DHEA) a kind of neurosteroid that decreases in the brain with aging.
Materials and Methods: In this study, neural progenitor cells obtained from mouse embryos' brain. Then, these cells were treated with 1μm concentration of DHEA for 48 hours. After 48 hours, the phosphorylation of GSK3β was analyzed by immunocytochemistry.
Results: The results of immunochemistry analysis showed that DHEA can increase phosphorylation of GSK3β in neural cells.
Conclusion: Overall, we conclude that phosphorylation of GSK3β by DHEA can help to cure AD with activation of Wnt signaling.
Keywords: Neural Progenitor Cells, Alzheimer Disease, Glycogen Syntase Kinase3β, Dihydroepiandrosterone

Ps-36: Expression of Drug Metabolism Enzymes in Hepatocyte-Like Cells Derived from Menstrual Blood Stem Cells

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Objective: Recently, valuable characteristics of menstrual blood stem cells (MenSCs) have impelled scientists to take its advantages for cell therapy of different diseases including liver disorders. In this study, we examined mRNA expression levels of drug metabolizing factors including glutathione S-transferase (GST) and cytochrome P- 450 (CYP) enzymes in differentiated hepatocyte-like cells from MenSCs.
Materials and Methods: The isolated MenSCs were characterized and differentiated into hepatocyte-like cells using hepatocyte growth factor (HGF) and oncostatin M (OSM) in combination with other components in serum-free culture media. After primary characterization of hepatocyte markers such as albumin (ALB) and tyrosine aminotransferase (TAT), mRNA expression of GSTA1, GSTA2, GSTP1, CYP3A4 and CYP7A1 were assessed in differentiated cells in reference to undifferentiated cells using real-time PCR.
Results: Based on immunofluorescent staining and Real-Time PCR data, the differentiated MenSCs could express hepatocyte markers like ALB and TAT at mRNA and protein levels suggesting development of hepatocyte-like cells from MenSCs. Moreover, the expression levels of GSTA1, GSTA2 and CYP3A4 mRNA were up-regulated in differentiated cells compared to undifferentiated cells. The expression of CYP7A1 gene was also remarkable on the last day of differentiation process. Although the expression level of GSTP1 also elevated during differentiation, it was not statistically significant (p=0.6).
Conclusion: Based on accumulative data, MenSCs could be viewed as an accessible population of stem cells with differentiation ability into drug-metabolizing hepatocyte-like cells.
Keywords: Menstrual Blood, Stem Cell, Hepatocyte, Differentiation

Ps-37: The Expression of NPPA Splice Variants during Cardiac Differentiation of Mouse Mesenchymal and Embryonic Stem Cells

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Objective: NPPA is an early and specific marker for functional myocardium of the embryonic heart. NPPA gene encodes for a precursor of atrial natriuretic peptide (ANP). The expression pattern of natriuretic peptide precursor A (NPPA) gene during development of cardiac chamber is highly dynamic. Moreover, the expression profile of NPPA gene is different between human and mouse. So far, three alternatively spliced variants have been reported for NPPA gene in human. In mouse, no alternatively spliced isoform of NPPA gene has been reported. In the current study, we studied the expression of NPPA gene during cardiac differentiation *in vitro* and in vivo.
Materials and Methods: Cardiac differentiation of the ADSCs was induced by 5-azacytidine, bone morphogenetic protein-4 (BMP4) or coculture with the mouse cardiomyocytes, and ES cells were differentiated at the presence or absence of bone morphogenetic protein-4 (BMP4). To assess the expression of NPPA splice variants during heart development, the cardiac area of 8-day mouse embryos and one-week old mice were isolated and used for evaluation.
Results: Two-week differentiated ADSCs and ES cells expressed some cardiac-specific makers, including atrial natriuretic peptide (ANP). Three additional intron-retained splice variants of NPPA were also detected during cardiac differentiation of the ADSCs and ES cells. In addition, we detected three intron-retained splice variants of NPPA in 8-day mouse embryonic heart. In the mature cardiomyocytes of 1-week old mice, only the correctly spliced isoform of NPPA gene was expressed. Freshly isolated stromal vascular fraction also expressed one intron-retained isoform of NPPA gene.
Conclusion: Our findings have provided evidence for the expression of intron-retained splices of NPPA mRNA during the early stages of mouse cardiogenesis as well as in the mouse adipose tissue. However, the functional significance of these variants remains to be investigated.
Keywords: NPPA, Splice Variants, ADSC, ES Cell, Cardiomyocyte

Ps-38: The Effects of Pre- and Post-Ischemic Neural Stem Cell Intra-Ventricular Injection on Brain Stroke in Rats

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Objective: Brain stroke, the second most important cause of mortality in developed countries, does not have

an effective treatment yet. Moreover, clinical outcome after stroke is not satisfactory and some patients remain disabled. In some cases such as surgeries, the patients face the risk of brain stroke. Nowadays, cell therapy is a new approach to establish a treatment for brain stroke. Neural stem cells are one of the good resources that can regenerate the central nervous system regarding their ability to differentiate into 3 neural lineages, including neurons, oligodendrocytes and astrocytes.

Materials and Methods: Neural stem cells were isolated from rat embryo, and then expanded until passage 4. The neural stem cells expressed Nestin and were differentiated to neurons, oligodendrocytes and astrocytes by adding 5% fetal bovine serum to their culture media. The isolated neural stem cells were labeled with PKH26 and injected into lateral ventricle of rat brains by stereotactic method in 2 groups: first group received the cells one day prior to brain stroke; the second one received the cells one day after the brain stroke. In control group, PBS was injected. HandE staining was performed to determine the necrosis and ischemic area.

Results: Neurological assessment shows that clinical improvement in second group is better than the first one after 28 days. Also, tracking the neural stem cells by PKH26 indicates that the cells show a preference to migrate more to the cortex and the striatum in comparison with the other areas of the brain in both groups. HandE staining expresses post-ischemic injection (2nd group) has the least necrosis and ischemic area and the control group has more ischemic and necrosis areas than the other groups.

Conclusion: Post-ischemic neural stem cell injection has better effects than pre-ischemic injection. Also, the injection of neural stem cells reduces the necrosis and the ischemic area according to the ability of these cells to regenerate the central nervous system.

Keywords: Neural Stem Cells, Ischemic Stroke, Brain, Regenerative Medicine

Ps-39: Quantitative Proteomics Analysis Highlights The Role of Redox Hemostasis and Energy Metabolism in Human Embryonic Stem Cell Differentiation to Neural Cells

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Objective: Neural differentiation of human embryonic stem cells (hESCs) is a unique opportunity for *in vitro* analyses of neurogenesis in humans. Extrinsic cues through neural plate formation are well described in the hESCs although intracellular mechanisms underlying neural development are largely unknown. Proteome analysis of hESCs differentiation to neural cells will help to further define molecular mechanisms involved in neurogenesis in humans.

Materials and Methods: Using a two-dimensional differential gel electrophoresis (2D-DIGE) coupled mass-spectrometry system, we analyzed the proteome of hESCs differentiation to neurons at three stages, early neural differentiation, neural ectoderm and mature neurons.

Results: Out of 137 differentially accumulated protein spots, 118 spots were identified using MALDI-TOF/TOF and LC MS/MS. We observed that proteins involved in redox hemostasis, vitamin and energy metabolism and ubiquitin dependent proteolysis were more abundant in differentiated cells, whereas the abundance of proteins associated with RNA processing and protein folding was higher in hESCs. Higher abundance of proteins involved in maintaining cellular redox state suggests the importance of redox hemostasis in neural differentiation. Furthermore, our results support the concept of a coupling mechanism between neuronal activity and glucose utilization. The protein network analysis showed that the majority of the interacting proteins were associated with the cell cycle and cellular proliferation.

Conclusion: These results enhanced our understanding of the molecular dynamics that underlie neural commitment and differentiation.

Keywords: hESC, Neural Differentiation, 2D-DIGE, Proteomics, Redox Homeostasis, Metabolism

Ps-40: Effect of Fndc5 Overexpression on Neural Differentiation Rate of mESCs

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Objective: Fndc5 has been recently recognized as a kind of myokine which could be cleaved and secreted as a hormone, termed Irisin, with an important role in thermogenesis and energy homeostasis. We have already indicated an increased expression of Fndc5 upon retinoic acid treatment during neural differentiation and knockdown of Fndc5 decreased neural differentiation and neurite outgrowth

Materials and Methods: We have established a

transduced cell line of mouse embryonic stem cells with ability to produce Fndc5 upon doxycycline induction. Neural differentiation was carried out simultaneously with induced expression of Fndc5.
Results: Data showed an enhancement in neuronal precursor markers and mature neurons marker upon inducing the overexpression of Fndc5, concluding that Fndc5 has an important role in neural differentiation.
Conclusion: Our data are in agreement with recent studies which implicate the proliferative role of Fndc5 on neural cells and increased expression of neurotrophins including BDNF
Keywords: Gene Expression, Fndc5, Neural Differentiation, Real-Time PCR

Ps-41: Synergistic Effects of High Doses of Thymoquinone on Tamoxifen Induced Apoptosis in Estrogen Receptor-Negative Cancer Cell Line

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Objective: The aim of this study was to evaluate Synergistic effect of Thymoquinone (The main active component of black seeds) with Tamoxifen drug on apoptosis of human breast cancer MDA-MB-231 cells (invasive and metastatic human breast cancer cell line, estrogen receptor negative).
Materials and Methods: The human breast cancer MDA-MB-231 cells were treated with Tamoxifen (2μM) separately or synergistic with Thymoquinone (150 μM). Morphological conformation of cell death in MDA-MB-231 cells treated by Tamoxifen, Thymoquinone and combination after 48hours was studied by TUNEL assay. The data were analyzed by One-Way ANOVA and Tukey's test. All statistical analyses were done using SPSS software (version19.0). In all cases, p values<0.05 was considered significant.
Results: The data of TUNEL assay in estrogen receptor-negative cancer cells after 48hours treatment were showed that Tamoxifen and Thymoquinone separately or synergistically significant rise of apoptotic index ($p<0.05$). This study demonstrates that Thymoquinone anti-tumor action is apart from estrogen pathway and combination of Thymoquinone and Tamoxifen induced extreme apoptotic index in estrogen receptor-negative cancer cells ($p<0.001$). So that higher dose of Thymoquinone (150 μM) in combination with lower dose of Tamoxifen (2 μM) induced apoptosis in MDA-MB-231 in a safe manner and free of side effects on normal cells.
Conclusion: Thymoquinone procedures through non-estrogen-dependent human breast cancer cells is induced apoptosis. Also, Thymoquinone will increase efficiency of Tamoxifen so we can using very low doses of tamoxifen acceptable amount of tumor cells destroyed. Induced apoptosis, the main mechanism of combination of Thymoquinone and Tamoxifen. These data could bring a new light for the treatment of resistant metastatic breast cancer and also for other type of cancers patients.
Keywords: Apoptosis, Breast Cancer, Tamoxifen, Thymoquinone

Ps-42: Expression Analysis of Two Stem Cell Markers, RHOXF2 and PIWIL2, in Acute Myeloblastic Leukemia Patients

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Objective: Acute myeloblastic leukemia (AML), the second most common adults' leukemia, has a high mortality rate. Therefore, researchers are investigating new treatments for this type of cancer. Cancer stem cell theory states that cancer originates from a subset of cells possess characteristics associated with normal stem cells. Cancer-testis (CT) antigens are a group of tumor associated antigens with restricted expression just in gametogenic tissues but expression in a wide variety of tumors. They are potential targets for cancer immunotherapy. Some of them are stem cell markers.
Materials and Methods: We analyzed expression of two previously known stem cell markers and CT antigens, RHOXF2 and PIWIL2, in 40 AML patients versus 10 normal donors by means of real time RT-PCR.
Results: RHOXF2 and PIWIL2 showed expression in normal leukocytes. Their expressions were not significantly different in normal and AML patients.
Conclusion: As these genes are expressed in normal leukocytes, their potential application in immunotherapy and as tumor biomarker is put under question. Future researches should focus on the expression profiles of so called CT antigens to find those with more testis specific expression.
Keywords: AML, Stem Cell Marker, RHOXF2, PIWIL2

Ps-43: Male and Female Rat Bone Marrow-Derived Mesenchymal Stem Cells Are Different in Terms of The Expression of Germ Cell Specific Genes

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Objective: Recent studies have shown that mesenchymal stem cells (MSCs), under appropriate conditions, can differentiate into cell types including germ cells (GCs). These studies also show that MSCs without any induction express some GC-specific genes innately. Moreover, one report suggests that female MSCs have greater tendency to differentiate into female instead of male GCs. Therefore, for the first time, this study attempts to assay and determine the differences between the expression levels of some important GC-specific genes (Stra8, Vasa, Dazl, Stella, Piwil2, Oct4, Fragilis, Rnf17 and c-Kit) in male and female bone marrow (BM)-MSCs of rats. Rats BM sampling was performed by a newly established method.
Materials and Methods: We cultured rat BM samples, then characterized male and female MSCs according to their adhesion onto the culture dish, their differentiation potential into bone, cartilage and fat cells, and phenotype analysis by flow cytometry. The expression of GC-specific genes and their expression levels were evaluated with reverse transcription polymerase chain reaction (RT-PCR) and real time RT-PCR.
Results: Our results showed that Dazl and Rnf17 did not express in the cells. The majority of examined genes, except the Piwil2, were expressed at almost the same levels in male and female MSCs. The expression of Piwil2 was significantly higher in male MSCs than the female cells.
Conclusion: A recently published study has shown that female mouse MSCs show greater tendency to differentiate into female germ cells. Here also, higher expression of Piwil2 would be probably related to the more prominent role of Piwil2 in the male GC development process. Male BM-MSCs appeared more prone to differentiate into male rather than female GCs. Additional research should be performed to determine the exact role of different genes in the male and female GC development process.
Keywords: Male and Female MSCs, Different, Expression, Germ Cell Genes

Ps-44: Effect of Rosiglitazon on Expression of Fibronectin Type III Domain-Containing 5 Protein and Cardiac Differentiation

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Objective: Fibronectin type III domain-containing 5 protein (Fndc5) is a type I membrane protein that has 209 amino acid residues. Bostrom et al. found that Peroxisome proliferator-activated receptor (PPAR) γ coactivator 1α (PGC1α) transcriptional co-activator expression induced Fndc5 expression in white adipose tissue (WAT) browning. Previous studies by our group have shown that Fndc5 expression increased during the process of cardiac differentiation of mouse embryonic stem cells (mESCs), leading us to conclude that it might be involved in cardiogenesis. Also we showed decreased PGC1α expression significantly reduced expression of Fndc5 and cardiac markers in cardiomyocyte.
Materials and Methods: In the present study, correlation between PGC1α and Fndc5 was examined by application of a PGC1α agonist (Rosiglitason) that increased PGC1α expression.
Results: Our results indicated that in the process of beating cardiomyocyte formation, Fndc5 and cardiac markers expression level was not influenced by PGC1α agonist significantly.
Conclusion: These results propose an idea that the ground state of PGC1α expression level is needed which needs further verification.
Keywords: Fibronectin Type III Domain-containing 5 Protein (Fndc5), PGC1α, Cardiac Differentiation

Ps-45: Evaluation of The VE-cadherin Gene Expression in Adipose Derived Stem Cells and Purified Mesenchymal Stem Cells under Chemical and Mechanical Stimuli

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Objective: Adipose-derived stem cells (ADSCs) are a heterogeneous cell population that may be enriched by positive selection with antibodies against CD271, yielding a selective cell universe with higher proliferation and differentiation potential. ADSC cells can sense their surrounding biochemical and biophysical factors, which play major roles in their differentiation toward different phenotypes. The scope of the current study is to evaluate and acquire the best endothelial differentiation yielding through purification of ADSCs using specific CD271 cell marker and compare their results with that of unpurified ADSCs both exposed under chemical

and mechanical conditions *in vitro*.Thus, we examined endothelial differentiation of neat ADSC and ADSC-CD271+ cells in response to specific growth factors, shear force and combination of these two factors.

Materials and Methods: MSCs were isolated from fat tissue and cultured in DMEM containing 10% FBS. Then they were characterized in passage 2, using flow cytometery. CD271 expression level was examined and purified using flow cytometery sorting. ADSCs-CD271+ were cultured and characterized and their expression level was examined. ADSCs and ADSCs-CD271+ were cultured in collagen coated-tubular silicone scaffolds and then exposed to VEGE (for 7 days), shear stress (in a designed perfusion bioreactor, under application of shear stress for 24 hours) and combination of these two signals. Real-Time PCR was used to quantify the expression level of the VE-cadherin gene (the factor specifying endothelial cells) in the experimental groups.

Results: 1% of ADSCs showed CD271 marker, which was increased to 74% after purification. Evaluation of surface markers using flow cytometry confirmed the mesenchymal characteristics of both cell groups. According to the results of Real-time PCR, the chemical-mechanical group, the mechanical group and then the chemical group (in both purified and unpurified cells) compared to their corresponding control group, showed the highest expression of VE-cadherin, respectively. Moreover, the expression of VE-cadherin was generally higher in ADSCs-CD271+ groups compared to results in ADSCs ones.

Conclusion: The findings of this study demonstrate that MSCs purification as well as combining the chemical and mechanical factors can improve VE-cadherin expression.

Keywords: Mesenchymal Stem Cell, CD271, VE-Cadherin gene, VEGF, Shear Stress

Ps-46: Umbilical Cord Blood-Derived CD133+ Stem Cells Generate Small Colony Forming Unit-Megakaryocyte *In Vitro*: A Hypothetical Reason for Tardiness in Platelet Recovery after Cord Blood Transplantation

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Objective: Although umbilical cord blood was considered as a waste material, now many cord blood banks save and prepare it for transplantation. The main concern following cord blood transplantation is tardiness of platelet recovery. One of the probable Feasons of this problem is incapacity to generate large colonies of megakaryocytic lineage. This hypothesis may be reasonable since larger colonies can produce more platelet.

Materials and Methods: In this study, we evaluated the colonogenic ability of CD133+ derived from cord blood in collagen-based medium *in vitro*. First, CD133+ cells were purified by direct and indirect magnetic sorting methods. Then the sorted cells were compared in terms of CD133, CD41, CD61, and CD42b expression by flow cytometric analysis immediately after purification and after 7 day culture in Stem Span serum-free-medium. Also, CD133+ and CD133- cells were seeded in collagen-based MegaCult-C medium containing recombinant human insulin, human transferrin, L-glutamine, 2-mercaptoethanol, rh TPO, rh IL-6, and rh IL-3 at 37°C and humidified condition for at most two weeks. According to manufacturer's instructions, colonies were stained by biotin-avidin conjugate for CD41 antigen and finally categorized in three groups: small, medium, and large.

Results: Our data showed that there was not any significant difference between direct and indirect method in expression of CD41, CD61, and CD42b in both after sorting and at day 7 of culture in Stem Span media. But it appeared that indirect method were more efficient to purify of CD133+ cells. In our experiments, CD133- cells could not generate any megakaryocytic colonies. CD133+ cells formed three categorized colonies though most of them were small.

Conclusion: Consequently, indirect magnetic method was more effective for purification of CD133+ cells. Since CD133+ derived from cord blood generated about 64% small colonies, it may convince delayed platelet recovery after cord blood transplantation.

Keywords: CD133+ Stem Cells, Umbilical Cord Blood, Platelet, Colony Forming Unit-Megakaryocyte

Ps-47: Platelet Lysate as An Alternative Supplement for Growth and Expansion of Human Bone Marrow Mesenchymal Stem Cells

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Objective: Despite the fact that there are huge capabilities for human mesenchymal stem cells (MSCs) to differentiate into various tissues, the major obstacle for using these cells in clinic is the presence of fetal bovine serum (FBS) that prohibits basically in human studies due to ethical issue and transferring animal diseases. For overcoming this drawback, many alternatives have been tested such as platelet lysate (PL).

Materials and Methods: Our main strategy was substation of animal sera using typically for cultivation of MSCs *in vitro* by umbilical cord blood-derived PL. In brief, two separate category of mesenchymal stem

cells was grown in MEM-alpha medium supplemented by either10% fetal calf serum or PL in ascending concentrations: 5, 10, and 20%. After >90% of confluence, mesenchymal stem cells were evaluated regarding surface markers, Adipo- and osteogenic differentiation, and cytotoxicity of PL. Also, doubling time and growth kinetics of two cell categories was calculated by a mathematic formula.

Results: MSCs in PL-supplemented medium have a significant expression of CD105, CD73, and CD29, but not in case of CD90, CD45/CD34, and CD31. PL groups have similar behavior compared with FBS group for differentiating toward adipocyte or osteocyte. Only 5% PL group were proliferated in parallel of FBS group with a bit longer doubling time while 10%, and 20% PL groups did not have any markedly expansion. In exception of 20% PL, we observed no cytotoxic effect for MSCs.

Conclusion: Platelet-derived products like as PL have been used widely for the expansion and differentiation of MSCs in clinical trials. However, it requires to test some different concentrations of PL for achieving the best compared with FBS. In the overall analysis, we found 5% PL obtained from umbilical cord blood can make a suitable serum-free media for MSCs will be used in the cell therapy centers.

Keywords: Mesenchymal Stem Cells, Platelet Lysate, Umbilical Cord Blood

Ps-48: Co-Transplantation of Pancreatic Islets and VEGF-Expressing Human Embryonic Stem Cell Derived Mesenchymal Stem Cells through Hydrogel to Ameliorate Alloxan Induced Diabetes in Nude Mice

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Objective: Pancreatic islet transplantation has emerged as a promising treatment for type I diabetes, a global health concern with increasing worldwide incidence of 3% per year. However, its efficacy is severely hampered due to poor islet engraftment and revascularization, which have been resulted to partially loss of transplanted islets. It has been shown that local delivery of vascular endothelial growth factor (VEGF) could accelerate transplanted islet revascularization, although permanent high level of VEGF may lead to undesirable side effects. In this study we investigated the effects of conditional cell-based delivery of VEGF through collagen-fibrin hydrogel on transplanted islet function and revascularization.

Materials and Methods: RH6 human embryonic stem cell derived mesenchymal stem cells (hESC-MSCs) have been transduced by two lentiviruses containing rtTA and VEGF-A. Tet-on expression of VEGF from these cells was shown by tube formation assay and was confirmed through VEGF ELISA. After co-transplantation of these cells and mouse isolated islets through collagen-fibrin hydrogel in the omental pouch of diabetic nude mice, the blood glucose, body weight, glucose tolerance and serum C-peptide was measured after 28 days. As control groups, islets were transplanted alone and with non-transgenic hESC-MSCs.

Results: Function of islets transplanted with hESC-MSC: VEGF in presence of Dox induction was significantly improved, accompanied with superior islet revascularization demonstrated by enhancement in micro-vessel density and area per vessel, compared with control groups.

Conclusion: We conclude that conditional expression of VEGF from hESC-MSCs during islet transplantation could enhance islet functionality and revascularization. This result can be used to improve the outcome of clinical islet transplantation.

Keywords: Embryonic Stem Cell, Mesenchymal Stem Cell, Islet, Revascularization

Ps-49: Dexamethasone, Low Intensity Ultrasound and Osteogenic Differentiation of Adult Stem Cells

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Objective: In this study, we investigated the combination effect of Dexamethasone (Dexa) and Low- Intensity Ultrasound (LIUS) on osteogenic differentiation of rat bone-marrow derived mesenchymal stem cell (rMSCs). Our hypothesis was synergistic effect of tow induction materials in osteogenic differentiation on rMSCs.

Materials and Methods: Ultrasound instrument with 3MHz frequency was calibrated by radiation force method. rMSCs isolated from rat's tibia and femur were cultivated. Cells promoted for three successive passages in order to purify them. Passage three rMSCs were grouped in 10-8mMol Dexa and LIUS and just Dexa. The LIUS and Dexa group recived stimulation

at 3MHa frequency and 355mW.cm-2, continues mod, for 5 minutes daily for 2 weeks. The control group just affected by Dexa. Alkaline phosphatase activity assay and semi-quantitative RT-PCR for alkaline phosphatase, Osteocalcin and Osteopontin expression in 1st, 3rd, 5th, 7th, 9th, 11th and 14th days post-stimulation, respectively.
Results: Our results showed that alkaline phosphatase activity in LIUS and Dexa group was significantly more than that of Dexa ($p \leq 0.005$). According to semi-quantitative RT-PCR data, differentiation genes expression was significantly different in tow groups ($p \leq 0.005$).
Conclusion: This study strongly suggests that Dexa and LIUS combination in osteogenic differentiation in rMSCs, was a synergistic effect and has a primitive effect on Osteogenic differentiation.
Keywords: Dexamethasone, Low-Intensity Ultrasound, Mesenchymal Stem Cells, Osteogenic Differentiation

Ps-50: Effects of Growth Factor and Shear Stress on The Stability of Differentiation of Mesenchymal Stem Cells, Based on The Expression of VE-cadherin Gene

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Objective: Mesenchymal stem cells have been proposed as a suitable cell source for vascular tissue engineering, because of their high proliferative capacity as well as their ability to differentiate into various phenotypes. Shear stress due to blood flow is one of the most important mechanical signals experienced by endothelial cells and can be used as a tool for inducing endothelial differentiation. The purpose of the present work is to examine the effects of endothelial growth factor (VEGF) and shear stress on the differentiation of mesenchymal stem cells into vascular endothelial cells and the stability of this differentiation.
Materials and Methods: Adipose-derived mesenchymal stem cells (AdMSCs), were isolated from human fat tissue, and characterized using flow cytometry. These cells were cultured in DMEM containing 20% FBS and passage 3 cells were used in experiments. AdMSCs were injected into collagen coated-tubular silicone scaffolds and exposed to proper culture conditions. Cells were exposed to VEGF (for 7 days), shear stress (for 24 hours) or both VEGF and shear stress. For studying the stability of differentiation, the mRNA levels of the VE-cadherin gene were quantified using Real-Time PCR, upon completion of each test, as well as on the 5th and 10th days after the test.
Results: According to the results, immediately upon the completion of tests as well as on the 5th day after the experiments, the mRNA level of VE-cadherin was higher in the mechanical group compared to the chemical and chemical-mechanical ones. But on the 10th day after the test, the highest expression was observed in the chemical-mechanical group.
Conclusion: According to the results, VE-cadherin gene expression was increased over time, in all three experimental groups, but this increase with time was more observable in the chemical-mechanical group. This can be due to the simultaneous effects of chemical and mechanical signals on the expression of endothelial genes in AdMSCs over time, compared to the effects of chemical or mechanical cues, alone.
Keywords: Mesenchymal Stem Cells, VE-cadherin Gene, Differentiation, Shear Stress, VEGF

Ps-51: Analysis of CX3CR Chemokine Gene Expression Involved in Homing in Primed Adipose Tissue Derived Mesenchymal Stem Cells with A Histone Deacethylase Inhibitor

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Objective: Human adipose tissue-derived mesenchymal stem cells (hAdMSCs) have the potential to restore the function of damaged tissues and, providing promising applications of tissue regenerative medicine strategies. CX3CR gene expression should increase the migratory potential of mesenchymal stem cells. However, the migratory efficiency of mesenchymal stem cells after transplantation becomes limited. To promote the process, treating these cells with valproic acid (VPA) as a histon deacetylase inhibitor can be helpful.
Materials and Methods: In this study, we isolated MSCs from adipose tissue. Next, we studied their characteristic functions. MSCs were accordingly isolated from heterogeneous cell populations. Then, 4 passages in culture were primed with VPA 5 mM for 24, 48 and 72hours. Detailed gene expression profiles were investigated using an RNA extraction, and subsequently a reverse transcription polymerase chain reaction (RT-PCR) analysis for cDNA synthesis. PCR was used to detect gene expression in MSCs treated and non-treated with VPA.
Results: The result showed that treatment with VPA

did not promote CX3CR expression in human Adipose derived mesenchymal stem cell. This indicate that VPA treatment could not stimulate migration and homing of mesenchymal stem cells.

Conclusion: However, using another concentration and/or different intervals might lead to an increased potential of these cells for cell-based therapies. Furthermore, these cells can be primed *in vitro* to enhance their *in vivo* recruitment for tissue repair.

Keywords: Mesenchymal Stem Cells, Migration, Homing, CX3CR, Valproic Acid

Ps-52: Analysis of CXCR4 Gene Expression in Primed Adipose Tissue Derived Mesenchymal Stem Cells with A Histone Deacethylase Inhibitor

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Objective: The migration and homing of mesenchymal stem cells to injured tissues is important for the correction of conditions such as neurodegenerative diseases. Human adipose-derived mesenchymal stem cells (hAdMSCs) have been used for transplantation and cell-based therapies. The multi-potential differentiation of these cells make them excellent options for future tissue engineering and repair. Valproic acid (VPA) is also known to inhibit histone deacetylases (HDACs) and cause diverse effects on mesenchymal stem cells. This study investigated whether treatment of MSCs with VPA would enhance the expression of CXC4 and cell migration. In this study, we isolated MSCs from adipose tissue. Next, we studied their characteristic functions.

Materials and Methods: MSCs were isolated from heterogeneous cell populations. After 4 passages in culture, the medium was primed with VPA 5 mM for 24, 48 and 72hours. Detailed gene expression profiles were investigated using a RNA extraction and subsequently a reverse transcription polymerase chain reaction (RT-PCR) analysis for cDNA synthesis. PCR was used to detect gene expression in MSCs treated and non-treated with VPA. The CXCR4 expression was observed in the VPA-treated group and control group at mRNA and protein levels.

Results: This result demonstrated that treatment with VPA greatly promoted CXCR4 expression in hAdMSCs. We also found that VPA significantly increased CXCR4 expression level in long-term treatments (72 hours). CXCR4 gene expression markedly increased in the VPA-treated groups compared to the control group.

Conclusion: These results showed that VPA could considerably improve the migratory potential of human adipose-derived mesenchymal stem cells. It was also found that VPA stimulated MSC migration and homing. Thus, these cells have the potential to be used for cell-based therapies.

Keywords: Mesenchymal Stem Cell, Migration, Homing, CXCR4, Valproic Acid

Ps-53: The Augmented BMP Pluripotency Pathway via TGF-β Suppression Maintains The Ground State of Embryonic Stem Cells Self-Renewal

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Objective: Embryonic stem cells (ESCs) are pluripotent cells with capacity for differentiation into all cell types that are most frequently established from the inner cell mass (ICM) of blastocysts. However, the efficiency of ESC generation is influenced by genetic background in mice; as some strains are recalcitrance to mESC line derivation. Recently, we identified the suppression of mitogen-activated protein kinase (MAPK) kinase (also known as MEK) and transforming growth factor β (TGFβ) type I receptors by PD0325901 and SB431542, respectively-the combination named as Royan 2 inhibitors or R2i- enables the highly efficient derivation of pluripotent mouse embryonic stem cells (mESCs) from different strains. The cellular and molecular analysis indicated that R2i supports the ground state of pluripotency in a different route from well-known 2i condition which the latter inhibits MEK and glycogen synthase kinase 3 (GSK3) by PD0325901 and CHIR99021, respectively.

Materials and Methods: To investigate the molecular basis by which R2i maintains pluripotency, microarray analysis was performed in two mESC lines which were simultaneously cultivated for ten times in 2i and R2i. Our data revealed the significant elevation of BMP4-associated genes in R2i-grown cells in comparison to 2i-grown cells. For functional pathway analysis, BMP4 signaling was inhibited in R2i- and 2i-grown cells by adding noggin (500 nM) or noggin (250 nM) plus dorsomorphin (5 μM), two potent BMP signaling inhibitors.

Results: We observed no significant changes in the morphology of ES cells and Oct4 expression in 2i-grown cells in the presence of the BMP4 signaling inhibitors, even after several passages. However, the self-renewal capability of R2i-grown cells was strongly and adversely affected over a brief time period

Conclusion: Our analysis highlighted BMP signaling as a pathway markedly induced by TGFβ inhibition in R2i-grown cells. Since several studies have indicated that BMP4 signaling through Smad1/5/8 suppresses developmental regulators such as neuroectodermal-associated genes and FGF signaling, we here demonstrated that R2i via activation of the 'differentiation-inhibiting' BMP4 signaling enhanced pluripotent state in mESCs even though this pathway seems to be dispensable in 2i culture condition.

Keywords: Embryonic Stem Cell, Pluripotency, Signaling Pathway, Small Molecule

Ps-54: Human Wharton's Jelly Mesenchymal Stem Cell Secretome Display Significant Antiproliferative Effect on K562 Leukemia Cells

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Objective: Leukemia accounts for one-third of all cancers diagnosed in children (ages 0 to 14 years) against existence of multiple current treatments such as chemotherapy and hematopoietic stem cell transplantation, mortality is high among patients. On the other hand sever adverse effects of current therapeutic plans remains a challenge. Accordingly research toward finding new therapeutic strategies has been continuing. One of the recently interesting areas in cancer research is mesenchymal stem cell therapy. However existing results show requirement for further experiments. Umbilical cord as a newly used source of mesenchymal stem cell should be investigated more before entering clinical cell therapy plans. In this regard we examined the effect of human Wharton's jelly mesenchymal stem cell secretome on K562 leukemia cells.
Materials and Methods: MSCs were first isolated from human umbilical cord, then characterized with respect to their morphology, surface antigenicity and mesodermal lineage differentiation potential. MSC secretome was collected and applied on K562 cells. BrdU proliferation assay and Annexin V-PI staining was used for K562 proliferation and apoptosis evaluations.
Results: Results displayed significant antiproliferative effect of Wharton's jelly MSC secretome on K562 leukemia cells.
Conclusion: MSC itself or even MSC secretome can be used as an anticancer agent in leukemia patients.
Keywords: Mesenchymal Stem Cells, Warthon's Jelly, Leukemia, Anticancer

Ps-55: Prestimulation of Human Wharton's Jelly Mesenchymal Stem Cells with IFNγ Do Not Change its Paracrine Effect on Cancer Cell Lines A549 and K562

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Objective: Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. Despite large volume of research and trials, there is still serious need for more effective and less toxic medicines. Mesenchymal stem cell therapy is a vastly growing area of research which is rapidly moving toward clinical applications including cancer therapy. In this regard two groups of results were obtained by scientist. First are studies which display anticancer effect of mesenchymal stem cells and others showed tumor promoting function of these cells. However parameters such as MSC source and tumor type are detrimental. In this study we are going to investigate if prestimulation of MSCs with IFNγ can make them active against cancer cell lines A549 and K562.
Materials and Methods: MSCs were first stimulated with 20 ng/ml IFNγ, then MSC secretome was collected and applied on tumor cells. Cell proliferation was measured by BrdU assay and apoptotic potential evaluated by Annexin V-PI staining.
Results: The effect of MSCs secreted materials on cancer cells did not change before and after MSC stimulation with IFNγ. In other words probable changes in MSC secretome due to IFNγ activation did not lead to changes of its anticancer function.
Conclusion: MSC prestimulation with IFNγ does not change its effect on cancer cell lines A549 and K562.
Keywords: Mesenchymal Stem Cells, Interferon Gamma, Anticancer Effect

Ps-56: Development of Biocompatible Microfluidic System for Neural Differentiation of Induced Pluripotent Somatic Stem Cells

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Objective: Controlling cellular orientation, proliferation and differentiation is useful in designing organ replacements and directing tissue regeneration.
Materials and Methods: In the present study, we focused on methods to produce microgrooves on biode-

gradable polymer surfaces, and the influences of physical guidance cues on directing alignment and spatially controlling the *in vitro* neural differentiation of human Induced pluripotent stem cells (hiPSCs) were studied. Scanning electron microscopy (SEM) imaging, quantitative real time RT-PCR (qPCR) and immunocytochemistry were used to analyze differentiated cells and their expression of nuronal-specific genes.

Results: Our observations confirmed the differentiation of hiPSCs to neuronal cells on microfluidic device and suggested their potential application in central nerve system regeneration.significant changes were observed on the neural gene expression with respect to the orientation.

Conclusion: According to our results we may conclude that the microfluidic system reported herein could be used as a potential cell carrier for neuronal tissue engineering and a combination of these systems and hiPSCs may have potential application in neuronal regenerative therapy.

Keywords: Microfluidic Device, Aligned Scaffold, Human Induced Pluripotent Stem Cells (hiPSCs), Neural Differentiation

Ps-57: MiR-214 and MiR-135 Have Conflicting Expression Pattern during C2C12 Myoblast to Myocyte Differentiation

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Objective: The process of generating muscle is highly complex and requires a broad spectrum of signaling molecules. MicroRNAs are evolutionarily conserved small RNAs that post-transcriptionally regulate gene expression and have emerged as critical regulators of skeletal muscle development.

Materials and Methods: In this study, mouse myoblast cells (C2C12) differentiation upon partial serum deprivation from myoblast cells into myocytes is confirmed by positive Immunocytochemistry for specific skeletal marker, myosin. Using Target Scan 6.2, miRwalk and RNAhybrid, we found that miR-214 and miR-135 targeted several signal molecules, which are regulator of myogenesis process and insulin pathway as such insulin receptor substrate 2 (IRS 2) and insulin receptor (INSR).

Results: After qRT-PCR analysis, we recognized miR-135 as a novel miRNA involved in skeletal muscle development which had different expression level during myogenic differentiation. According to our data, miR-135 was up-regulated but miR-214 expression was down-regulated in myocytes. Interestingly, it was shown that the expression level of IRS-2 and INSR, were up-regulated in differentiated cells in comparison with undifferentiated one.

Conclusion: Using a bioinformatics approach, we identified IRS-2 and INSR, as targets of miR-214. Decreased expression of miR-214 in myocytes in comparision with myoblasts was accompanied with overexpression of IRS-2 and INSR, leading to myogenic differentiation progression. According to previous studies, it has been reported that muscle differentiation is blocked by decreased IRS1/2 and PI3k activity. Interestingly our study demonstrated that down-regulation of miR-214 may accelerate myogenesis because of increasing its target mRNA, IRS-2 during myogenic differentiation. In summary, our data identifies miR-214 and miR-135 as potential regulator of myogenesis through regulation of IRS/PI3K pathway.

Keywords: MicroRNA, C2C12 Differentiatin

Ps-58: Study of Differentiation Potency of Several Mouse iPSC Clones, Generated by Doxycycline Inducible Lentiviral Transduction System, into Definitive Endoderm Using IDE1 Molecule

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Objective: Regarding the pluripotency of induced pluripotent stem cells (iPSCs), i.e. no immunological rejection and no ethical issues, iPSCs are considered as an unlimited cell source for potential cell-replacement therapy. Definitive endoderm formation is one of the important steps in the development of organs like pancreas and liver. Because efficiently derived definitive endoderm would potentially serve as an important source for liver and pancreas cell therapy, introducing more efficient protocols for producing definitive endoderm is therefore of great importance.

Materials and Methods: In this study, the differentiation potency of three different iPSC clones toward definitive endoderm were assessed. The small molecule IDE1, introduced in 2009 by Melton et al. for definitive endoderm differentiation, was used in this process. Definitive endoderm markers were confirmed using real-time PCR and ICC.

Results: The Result of this study showed an increase in the expression level of the endoderm markers SOX17 and GSC and a decrease in the expression level of the ectoderm marker SOX1 after treatment with IDE1.

Conclusion: This study indicates that IDE1 promotes definitive endoderm formation more efficiently in comparison with the untreated groups. Among the iPSC clones studied, one clone showed more similarity to the

embryonic stem cell (ESC) control in differentiation capability which may be due to the genetic backgrounds and its more similarity to ESC control.
Keywords: Definitive Endoderm, iPSCs, Small Molecule, IDE1

Ps-59: Chemotactic and Invasive Role of Galectin-3 in Ovarian Cancer

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Objective: Galectin-3 (Gal-3) binds -galactosides through carbohydrate recognition domain, involving in various processes such as cell proliferation, apoptosis, cell adhesion, invasion, and metastasis. However its role in ovarian cancer remains unknown. The present study sought to determine Gal-3 role in invasion and migration of human ovarian cancer cell line SKOV-3.
Materials and Methods: SKOV-3 cells were seeded on the top of transwell inserts (25×10^4 Cells/ well) in the serum free media and lower compartment contained media with 10% FBS. Recombinant human Gal-3 (rh-Gal-3) at 3.3 μM was either added in the upper compartment or in lower compartment alone without serum. In parallel, 0.1% PectaSol-C modified citrus pectin (Pect-MCP) (a competitive inhibitor of Gal-3) was added to the upper compartment in combination with rhGal-3. Invasion assay was performed with matrigel transwells. Migration and invasion were assessed after 5 hours or 20 hours, respectively.
Results: There was 2.85-fold increased invasion ($p<0.001$) whereas, migration was decreased by 53% ($p<0.01$) in the presence of rhGal-3 compared to untreated cells. Moreover, Pect-MCP in combination with rhGal-3 abrogated invasive effect of rhGal-3. However combination of Pect-MCP and rhGal-3 did not change inhibitory effect of rhGal-3 on cell migration. Interestingly, rhGal-3 in the lower compartment increased cell migration by 7-fold.
Conclusion: This study for the first time demonstrates Gal-3 involvement in ovarian cancer invasion and migration. Moreover, our data showed that Gal-3 may be involved in chemotaxis. Thus, further investigation on the role of Gal-3 may warrant its use as a potential therapeutic tool in ovarian cancer.
Keywords: Galectin-3, SKOV3, Migration, Invasion, Chemotaxis

Ps-60: Randomized Double Blind Clinical Trial: Utilization of Umbilical Cord Blood-Derived Platelet Gel for Treatment of Diabetic Foot Ulcers

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Objective: Diabetes is a multiorgan disease and considered a major health problem in different societies. One of the complications is the pain, particularly in the extremities resulting from a process known as diabetic foot ulcer. The diabetic patients are subjected to many complications because of foot ulcers, many of them similar to chronic wound disease or pressure ulcers (bed sore).. Among many tested materials and works for wound healing such as debridement, tissue oxygenation, and skin transplantation, platelet-derived compounds are allocated the pivotal position by investigators for tissue regeneration and shortening the wound healing process. Many platelet components are procured from platelet rich plasma (PRP) from whole blood donation. An additional purification step is required to diminish the volume and facilitate handling in some studies. It means that platelet concentrates (PCs) may be obtained leading to more concentrated platelets in lower volume. For better efficacy and comfortable utilization of the platelet, it is feasible to form the platelet gel and then apply on wound sites. The aim of this trial was to show the efficiency of platelet derived-cord blood on healing diabetic ulcers (wound reduction, rates of complete healing, infection prevention, Osteomyelitis) versus placebo (the standard care only)
Materials and Methods: This study is a double blind randomized controlled trial to evaluate the positive effects of umbilical cord blood-derived platelet gel in 244 patients with diabetic foot ulcers. In this study all qualified patients (based on the inclusion and exclusion criteria) were randomly allocated into three study groups by a Stratified Permuted Block randomization method: group Received platelet rich plasma gel, group B (placebo) received platelet poor plasma gel, and group C received lubricant gel. Group A (interventional): application of 20-30 mL of gel from platelet rich plasma (PRP) Group B (placebo): application of 20-30 mL of gel from platelet poor plasma (PRP) Group C (control): application of 20-30 mL of lubricant gel (used typically for sonography) All patients received weekly application above mentioned gels for 3 month (intervals: 1 week).
Results: In this study we did not observe any significance among three therapeutic groups in wound recovery and tissue regeneration. In other words the platelet gel group demonstrated the same efficacy in comparison with platelet poor plasma (PPP) gel and the placebo group ($p> 0.05$).
Conclusion: Although our results showed that growth factors in platelet granules may help the wound healing process and tissue regeneration, there are other factors that should be considered precisely for a statistically better clinical outcome. Another possible reason for not achieving the desirable effect is probably our diabetic

patients did not respond sufficiently because of pathologic or genetic conditions.
Keywords: Platelet Gel, Diabetic Foot Ulcer, Umbilical Cord Blood, Wound Healing

Ps-61: Isolation, Expansion, and Differentiation of Human Fetus Hippocampus Neural Stem Cells

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Objective: Neural stem cells (NSCs) are the only cells that are capable to regenerate the central nervous system (CNS) except embryonic stem cells. These cells are achievable from different zones in CNS such as sub-ventricular zone, hippocampus, epandymal cells, etc. Neural stem cells have the ability to differentiate to neuron, oligodendrocyte and astrocyte. Also, NSCs create a new approach to treat some neurodegenerative diseases such as Parkinson, Alzheimer and the other CNS disease including brain stroke and spinal cord injury. In this study we have decided to isolate, expand and differentiate human fetus hippocampus neural stem cells to three neural lineages.
Materials and Methods: Tissue samples were dissected from hippocampus of legal aborted 20 week human fetus. Each sample was washed 3 times with cold PBS then the trypsin was added and incubates for 5 minutes. To inactive the trypsin, trypsin inhibitor was added to the sample. The isolated cells were cultured in neurobasal media containing B27, bFGF 10 µg/ml and EGF 20 µg/ml. To differentiate the NSCs into three neural lineage cells fetal bovine serum (5%) added to the media.
Results: The isolated cells could form some spheres after 5 days called neurospheres and these cells express Nestin. Differentiated cells were fixed and there were some cells expressed β-tubulin (neuron marker), some of them GFAP (astrocyte marker) and a few of them O4 (oligodendrocyte marker).
Conclusion: Our study shows that there are some neural stem cells in hippocampus of human fetus which are able to provide three different CNS cells and it could be a good choice for treating CNS disease.
Keywords: Neural Stem Cells, Hippocampus, Human Fetus

Ps-62: Spinal Cord Is An Appropriate Source of Neural Stem Cells

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Objective: In the future a reliable approach for management of neurodegenerative diseases will be cell therapy and using the neural stem cells. One of these diseases is spinal cord injury that caused by trauma in adult male and is one of the most important cause of disability. Neural stem cells isolated from same niches could make better connection and signaling than the cells from other sources. Here, in this study we isolate neural stem cells from adult human spinal cord and human fetus spinal cord.
Materials and Methods: For isolating neural stem cells from human adult and fetus spinal cord, the samples were collected from aborted fetus with gestational age 20 week (for human fetus sample) and traumatize spinal cord (for adult samples). after washing with phosphate buffer saline (PBS) containing 15% pen/strep, the trypsin was added for 5 minutes. Then trypsin inhibitor was added to samples and the suspension passed through cell strainer 70µm.the samples centrifuged for 5 minutes with 110g.the isolated cells were cultured in DMEM/F12 containing 2% B27, 1% N2, 1% pen/strep, 20 ng/ml Epidermal growth factor (EGF) and 10 ng/ml basic fibroblast growth factor (bFGF).For differentiation of these cells to neurons, oligodendrocytes and astrocytes the growth factor were removed and 5% fetal bovine serum was added to their media.
Results: After 1 week the isolated cells made some sphere, the sphere forming frequency was stable till passage 10. Also,the isolated cells expressed Nestin and CD133 (markers for neural stem cells). Differentiated cells express β-tubulin and MAP2 (markers for neuron), O4 (oligodendrocyte marker) and GFAP (astrocyte marker).
Conclusion: Both human adult and fetus spinal cord are appropriate sources for isolating neural stem cells and using them for spinal cord injury cell therapy; the isolated cells are capable to differentiate to three neural lineage cells (neurons, oligodendrocytes and astrocytes).
Keywords: Neural Stem Cell, Spinal Cord, Differentiation

Ps-63: Differentiation of Wharton's Jelly Mesenchymal Stem Cells to Neuron and Motor Neuron in Alginate Scaffold

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Objective: Microenvironment affects the cells' behavior in different cell types including Mesenchymal Stem Cells (MSCs) by its mechanical properties. The elasticity of the micro-environment in three dimensional (3D) scaffold change the viability, proliferation and differentiation of MSCs. Alginate is a poly saccharine that could be used as a 3D scaffold and its elasticity changes with different concentrations. MSCs could be differentiated to neuron in alginate scaffold and this differentiation would be more in some concentrations than the others. Our goals in this study were to identify the differentiation of MSCs into neurons and motor neurons in the best concentrations for MSCs' viability and proliferation according to our previous study.
Materials and Methods: Whartons'Jelly Mesenchymal stem cells were obtained from fresh umbilical cord after the blood vessels removed and addition of collagenas. The isolated MSCs were cultured in DMEM containing 10% fetal bovine serum and 1% pen/strep. MSCs were positive for CD44. CD105, CD106, and CD90 and negative for CD34 and CD45.Their multipotency was confirmed by differentiating to adipocyte and osteocyte. For neural differentiation the MSCs cultured in 0.25% alginate and 50mM $CaCl^2$ (the optimum concentration for MSCs proliferation and viability) and the retinoic acid 10 µM and basic fibroblast growth factor (bFGF) 20 ng/ml were added to their media. After 1 week, the immunofluorescence staining was performed on the cultured cells in alginate scaffold for β-tubulin (a marker for neuron) and CD271 (motor neuron marker).
Results: The expression of β-tubulin and CD271 are more in this 3D alginate scaffold concentration than 2D culture (P value≤0.05).
Conclusion: The physical profile of alginate scaffold and its elasticity induces more MSCs to differentiate to neurons and motor neurons. This study indicates that there are some modalities such as the pressure effects of the MSCs' surrounding area caused by 3D scaffold could affect neural and motor neuron differentiation from Whartons' Jelly mesenchymal stem cells
Keywords: Whartons' Jelly Mesenchymal Stem Cell, Neuron, Motor Neuron, Alginate

Ps-64: Comparison of Immunomodulatory Biomolecules Secretion by Mesenchymal Stem Cells Derived from Murine Lung and Adipose Tissues

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Objective: Mesenchymal stem cells (MSCs) are a unique subset of progenitor cells and therapeutic stem cell sources for regeneration of damaged tissues. These tissue resident stem cells have a potential to regulate the immune system by secretion of soluble factors including transforming growth factor-β (TGF-β) and nitric oxide (NO). MSCs have been disclosed in lung and adipose tissues but the comparison of adult lung and adipose tissue resident MSC populations is still unclear. In the present study, TGF-β and NO secretion were compared in isolated MSCs from lung and adipose tissues.
Materials and Methods: MSCs were isolated from murine lung and adipose tissues and then cultured. Following proliferation, specific differentiation media were added for differential investigations and the expression of a set of surface antigenic proteins, CD73, CD105, CD90, CD34, CD45 and CD11b, were measured and compared in both cell populations. Finally, TGF-β and NO productive levels were assayed and compared in cultured lung-derived MSCs and adipose tissue-derived MSCs populations.
Results: Both stem cell populations revealed fibroblastoid morphology with similar size. MSCs from lung and adipose tissues expressed common positive markers; CD73, CD105 and CD90. In addition, they showed low levels of CD34, CD45 and CD11b. The differentiation of adipocyte and osteocyte were detected by two MSC populations. Eventually, no significant difference was statistically found in TGF-β and NO production between lung-derived MSCs and adipose-derived MSCs.
Conclusion: According to the similarity of lung-derived MSCs and adipose tissue-derived MSCs in immunophenotypes and secretions, it is proposed that adipose-derived mesenchymal stem cells could be an alternative stock of stem cells for lung cell therapy and transplantation.
Keywords: MSCs, Adipose, Lung, TGF-β, NO

Ps-65: Mesenchymal Stem Cells into Germ Cells

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Objective: Due to problems regarding use of embryonic stem cells (ESCs), many novel sources along with

various cultures have been investigated hoping to find a proper alternative. As the objective of the current study, we present the efficacy of a combined medium, retinoic acid together with BMP4, on differentiation process of bone marrow derived mesenchymal stem (BMDMSC) into germ cells.

Materials and Methods: To find differentiation capability, after providing purified BMDMSCs, differentiation to osteoblast and adipocyte was confirmed by using appropriate culture medium. To confirm mesenchymal lineage production superficial markers (expression of CD90 and CD73 and non-expression of CD45 and VEGFR2) were investigated by flowcytometry. Then the cells were differentiated to germ cells in mediums containing Retinoic Acid for 7 days and then BMP4 for 4 days. To evaluate germ cells characteristic markers (Mvh, Dazl) flowcytometry and immunoflourescence were used.

Results: After 4 passage BMDMSCs were fibroblast-like cells.The cells were able to differentiate into osteoblast and adipocyte cells. Presentation of stem cell superficial markers and absence of endothelial and blood cell markers were confirmative for mesenchymal origination of these cells. This fact was representative for multipotential entity of the examined cells. The flowcytometry and immunoflourescence results showed expression of germ cells characteristic markers.

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

Keywords: Mesenchymal Stem Cells, Infertility, Germ cells, BMP4, Retinoic Acid

Ps-66: Survey of Interaction between Induced Pluripotent Stem Cells and PLA/ Gelatin Scaffold

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Objective: Today, induced pluripotent stem cells (iPSCs) have been recognized as a new and good cell source for cell therapy. In recent years, research in the field of cell therapy and tissue engineering is widely developed and today there is a potential for the preparation of various types of scaffolds for different cells. In this study, in order to increase surface adhesion properties and cell survival, nanofiber scaffold polylactic acid was modified by using suitable concentration of gelatin.

Materials and Methods: Different scaffolds were fabricated by an electro spinning technique and cells morphology and cells viability were evaluated by using a scanning electron microscopy and MTT assay, respectively.

Results: The results of this study demonstrated that the modified PLA/gelatin scaffold is a more appropriate model for the adhesion, proliferation and survival of iPS cells. PLA/gelatin scaffold significantly increases cell attention and cell survival.

Conclusion: These findings are an important step in the use of appropriate three dimensional culture in order to treat a variety of diseases.

Keywords: PLA, Gelatin, iPS Cells, Electrospun Scaffold, Adhesion and Viability

Ps-67: Production of Electrospun PLA/ Gelatin Scaffold for Skin Tissue Engineering

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Objective: Repair or replacement of damaged tissues by using tissue engineering technology is considered to be a fine solution for enhanced treatment of different diseases such as skin diseases. Although the nanofibers made of synthetic degradable polymers such as polylactic acid (PLA), have been widely used in the medical field, they do not favor cellular adhesion and proliferation. In order to enhance cell adherence on scaffold and improve biocompatibility, in this study, the surface of PLA scaffold was modified by gelatin.

Materials and Methods: For electrospinning, PLA and gelatin were dissolved in hexafluoroisopropanol (HFIP) solvent at varying compositions (PLA:gelatin at 3:7 and 7:3). The properties of the blending nanofiber scaffold were investigated by fourier transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM). HandE and Masson̓ s trichrome staining methods were used to histological analysis.

Results: Our results showed that the modified PLA/ gelatin 7/3 scaffold is more suitable for fibroblasts attachment and viability than the PLA or gelatin nanofiber alone and fibroblast cultured on PLA/gelatin scaffold is suggested as an alternative way to improve skin wound healing.

Conclusion: The present study developed a simple but efficient modification to PLA scaffolds enhancing the potential application of PLA scaffolds in the field of skin tissue engineering applications and wound healing.

Keywords: Electrospinning, Gelatin, PLA, Skin Tissue Engineering

Ps-68: The Effect of PLA/CS Scaffold in Improving Skin Wound Healing

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Objective: Although chronic skin wounds are common, treatment for these disabling conditions remains limited and largely ineffective. In this study, we examined benefits of transplantation of fibroblast cells, cultured on poly lactic acid/chitosan (PLA/CS) scaffold, in wound healing.
Materials and Methods: In this study PLA/CS scaffold was prepared by electrospinning method then fibroblast cells were cultured on nanofibrous PLA/CS scaffold then transplanted in rat model. Histological staining methods were used to evaluate cell density, blood vessels and amount of collagen.
Results: The macroscopic observations showed wound more quickly improved in scaffold with cells group and slowly improved in control groups. In vivo assessment showed that treatment with fibroblast cell loaded scaffolds significantly promoted blood vessels formation, cell density and amount of collagen in rat compared with the control groups.
Conclusion: These results indicated the capacity of nanofibrous PLA/CS scaffold cultured with fibroblast cells to skin wound healing in vivo.
Keywords: Fibroblast Cells, Nanofibrous Scaffold, Skin Wound

Ps-69: Neuronal Class Determination: It Is All about Design and Equilibrium

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Objective: During differentiation a regular change in cellular biochemistry and function gives rise to specialized cell types. The process of differentiation is generally divided into two stages: specification and determination. At specification stage, the commitment is still capable of being reversed. But determination is a multistep and usually but not always irreversible process. It has been proposed that terminal selector genes define assets of neuron classes without touching overall neuronal identity and are, therefore, determinants of neural stem cells. Another feature of determination is robustness in the neuron regardless of changes in the internal and external environment.
Materials and Methods: In this study, we address the components of the odorant receptor cis-regulatory regions required for robust odorant receptor expression in Drosophila by the use of bioinformatics and applying different methods like immunohistochemistry and real-time PCR and strong genetic tools in Drosophila like RNAi gene knockdown.
Results: Our results show that short clusters with only a few required DNA binding motifs are sufficient to produce expression specificity. These clusters use structural components as motif overlap, order and proximity to provide the specificity. We also show that terminal selector genes are not only the last determinants of olfactory sensory neurons but also are essential for the robust expression of the olfactory receptors. We further demonstrate that the motif cluster for Or59b does not maintain expression if the metabolism or temperature changes. Robustness is moreover produced by the addition of solitary motifs found in the cis-regulatory region of the odorant receptors and the number of these motifs defines the expression strength and robustness of the odorant receptors.
Conclusion: Taken together our results support an additive model of the cis-regulatory region of odorant receptors whereby motif clusters permit expression and the number of solitary motifs provide expression level and robustness of the odorant receptors.
Keywords: Drosophila, Olfactory System, Robustness, Cell Determination

Ps-70: Epigenetic Alteration by DNA Promoter Hypermethylation of Vitamin D Receptor in Multiple Sclerosis Patients

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Objective: Multiple sclerosis (MS) is a neurodegenerative autoimmune disease characterized by recurrent episodes of demyelination and axonal injury mediated primarily by CD4+ T-helper cells with a pro inflammatory Th1 phenotype, macrophages, and soluble mediators of inflammation. Calcitriol (1α, $25(OH)_2$-Vitamin D3) binds to the vitamin D receptor (VDR) and regulates differentiation of the immune cells, and may therefore be useful in the treatment of autoimmune diseases as an immunomoderator drug. Vitamin D3 clinically used to moderate patient's symptoms. But in some patients no clinical improvement had been observed any more. Nowadays, scientists' attention is focused on epigenetic alteration such as differences in CpG dinucleotide methylation of some disorders like Arthritis Rheumatoid, Lupus Erythematous, different type of cancers and so. VDR binds to Vitamin D3 and RXR (Retinoic acid receptor) and this heterodimer binds to VDRE (VDR Element) upstream of target genes. The aim of the study was to determine whether is the VDR promoter methylation cause of its low expression in those group that doesn't respond to Vitamin D3 therapy or not.

Materials and Methods: We used bisulfite converted DNA, which extracted from 15 cases of two groups –which respond and doesn't respond to Vitamin D3- and converted by ZymoResearch bisulfite conversion kit, and specific primers for three potential regions of the VDR CpG island to perform Methylation Specific High Resolution Melting analysis (MS-HRM).
Results: Our analysis shown different patterns between two groups. Further, our data confirmed by DNA sequencing.
Conclusion: Promoter hypermethylation in CpG dinucleotides can directly affect and reduce the expression of VDR, so we concluded that vitamin D treatment could have an impact on epigenetic mechanisms of gene regulation.
Keywords: Multiple Sclerosis, Epigenetics, Vitamin D Receptor

Ps-71: Decreased Promoter Methylation of Interleukin-10 Gene in Leukocytes of Multiple Sclerosis Subjects

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Objective: Multiple sclerosis (MS) is a neurodegenerative autoimmune disease characterized by recurrent episodes of demyelination and axonal injury mediated primarily by CD4+ T-helper cells with a pro inflammatory Th1 phenotype, macrophages, and soluble mediators of inflammation. Calcitriol (1α, 25(OH)2-vitamin D3) binds to the vitamin D receptor (VDR) and regulates differentiation of the immune cells, and may therefore be useful in the treatment of autoimmune diseases as an immunomoderator drug. Vitamin D3 clinically used to moderate patient's symptoms. But in some patients no clinical improvement had been observed any more. Nowadays, scientists' attention is focused on epigenetic alteration such as differences in CpG dinucleotide methylation of some disorders like Arthritis Rheumatoid, Lupus Erythematous, different type of cancers and so. VDR binds to Vitamin D3 and RXR (Retinoic acid receptor) and this heterodimer binds to VDRE (VDR Element) upstream of target genes.The aim of the study was to investigate whether the vitamin D could affect epigenetic alteration of IL-10 promoter and increase its expression level in positive groups with positive response to Vit D3 therapy.
Materials and Methods: We extracted DNA from 15 cases of two groups –which respond (positive) and doesn't respond to Vit D3- and converted it by ZymoResearch bisulfite conversion kit. Specific primers was designated for potential region of the IL-10 promoter to perform Methylation Specific High Resolution Melting analysis (MS-HRM).
Results: Our analysis shown different patterns between two groups. Further, our data confirmed by DNA sequencing.
Conclusion: Promoter hypomethylation in CpG dinucleotides can directly affect and increase the expression of IL-10, so we concluded that vitamin D treatment could have an impact on epigenetic mechanisms of gene regulation.
Keywords: Multiple Sclerosis, Epigenetics, Interleukin-10

Ps-72: Co-Culture with Embryonic Stem Cells Improves Neural Differentiation of Adipose Tissue-Derived Stem Cells

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Objective: Neural differentiation of embryonic and adult stem cells has been reported previously. Although embryonic stem (ES) cells have an excellent potential for differentiation, their clinical application has been confronted by several problems including immune rejection, tumorigenicity and ethical issues. To overcome these problems, transplantation of autologous adult stem cells can be implemented as a more practical and feasible choice. During recent years, adipose tissue has been identified as an accessible and rich source of stem cells with multipotential differentiation capacity. So far, several research groups have reported neural differentiation of adipose tissue-derived stem cells (ADSCs) in low-serum or serum-free media with a cocktail of neural inducing factors. In the present study, we evaluated the effectiveness of a medium containing a synthetic serum replacement (KOSR) for neural differentiation of mouse ADSCs, and compared this medium with low-serum condition. Moreover, we evaluated neural differentiation of the ADSCs following indirect co-culture with ES cells.
Materials and Methods: ADSCs from the inguinal adipose tissue of 8-10-weeks old NMRI mice were isolated using 2 mg/ml collagenase A and were characterized using flow cytometry. At first, neural differentiation of the ADSCs was induced under two different culture conditions, DMEM plus 4% FBS and DMEM plus 15% KOSR, with or without β-ME. Then, third-passaged ADSCs were indirectly co-cultured with ES cells, and the expression levels of pluripotency markers, Oct4 and Sox2, mesenchymal stem cell markers, CD and CD, and proliferating cell nuclear antigens (PCNA) were assessed in the co-cultured ADSCs. Moreover, the control and co-cultured ADSCs were differentiated to neuron with or without RA treatment.
Results: Our findings showed that medium containing KOSR without any additional factor induces neural differentiation of the ADSCs. Two weeks differentiated ADSCs expressed several neuron-specific markers, and

RA treatment improved neural differentiation of the ADSCs. The expression levels of Oct4, Sox2 and PCNA were upregulated in the co-cultured ADSCs. Moreover, coculture with the ES cells significantly improved neural differentiation of the ADSCs. Treatment of the co-cultured ADSCs with RA diminished the expression of neural maturation markers.

Conclusion: Our findings are indicating that mouse ADSCs are capable of neural development in medium containing KOSR. Moreover, co-culture with the ES cells efficiently improves neural differentiation of the ADSCs. Probably, secretion of cytokines, chemokines, interleukins and some growth factors by ES cells have a positive effect on the late maturation of ADSC-derived neurons. Non-contact co-culture with the ES cells may be used as an efficient strategy to improve differentiation potential of adult stem cells for developmental studies and regenerative medicine.

Keywords: ADSC, Embryonic Stem Cell, Co-culture, Differentiation, Neuron

Ps-73: The Study of Bone Marrow Stromal Cells Cultured on PLGA Nanofibers

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Objective: The most important factor in choosing a polymer for the preparation of nano-fiber for use in medical and biological purposes, having the properties of biocompatibility and biodegradation. PLGA for the above benefits and much attention in tissue engineering, the present study aimed at examining bone marrow stromal cells cultured on PLGA nanofibers have been consideration.

Materials and Methods: In this study, the PLGA nanofiber by electro spinning technique with solvent hexa fluoro propanol was prepared. For study Properties of the polymer using scanning electron microscopy (SEM) and were analyzed invert microscope. After harvesting the BMSCs rat cells to achieve passage of two, cells in two groups PLGA nanofibers and without nanofibers were cultured. Cell proliferation on day's two, four and six by Acridine Orange and morphology of cells was examined by SEM.

Results: The results showed significant reduction in cell proliferation in PLGA nanofibers alone ($p<0.05$).

Conclusion: According to the survey results of BMSc cell culture, we can conclude that PLGA nanofibers can be used as biodegradable and biocompatible scaffold with bone marrow cells used in tissue engineering.

Keywords: Nano Fiber, PLGA, Stromal Cells in the Bone Marrow, Tissue Engineering, Cell Culture

Ps-74: Transplantation of Islet-Like Cell Clusters Derived from Human Dental Pulp Stem Cells Restores Normoglycemia in Diabetic Mice

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Objective: The success of islet transplantation for diabetes depends on the availability of an adequate number of islets, allogeneic or autologous origin. Post natal stem cells are now considered for the generation of physiologically competent, insulin-producing cells.

Materials and Methods: In this investigation, we generate functional islets from stem cells from pulps of human exfoliated deciduous teeth (SHED) and permanent teeth (DPSCs) by using a serum free cocktail of three-step protocol and then compared the yield of generated islet like cell clusters (ICCs) from SHED and DPSCs. We, packed ICCs derived from SHED in immuno-isolatory biocompatible macro capsules and then transplanted into Streptozotocin (STZ) induced diabetic mice. Non diabetic and diabetic controls were run parallel to the experimental diabetic group transplanted with macro capsules with or without islets.

Results: We found SHED superior to DPSCs. We observed that STZ diabetic mice alone and those transplanted with empty macro capsules exhibited hyperglycemia throughout the experiment whereas those transplanted with macro capsules containing ICCs restored normoglycemia within 3-4 weeks which persisted for more than 60 days.

Conclusion: ICCs derived from SHED upon transplantation to STZ diabetic mice can bring about reversal of experimental diabetes by two ways (i) supply of exogenous insulin secreted by ICCs and (ii) triggering of pancreatic regeneration via paracrine secretions of ICCs. Our results demonstrate for the first time that ICCs derived from SHED could reverse the STZ diabetes in mice without immunosuppression and offer an autologous and non-controversial source of human tissue that could be used for stem cell therapy in diabetes.

Keywords: Diabetes, Human Postnatal Dental Pulp Stem Cells (DPSCs), Islet-Like Cells Clusters (ICCs), Stem Cells from Human Exfoliated Deciduous Teeth (SHED), Transplantation

Ps-75: ProMyelocytic Leukemia (PML) Expression during Human Embryonic

Stem Cells (hESCs) Neural Differentiation Indicates A Role for PML Nuclear Bodies (PML-NBs) in Cellular Pluripotency Than Neural Differentiation

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Objective: Pro-myelotic leukemia (PML) is one of the major proteins in promyelotic leukemia nuclear bodies (PML-NBs). Retinoic acid (RA) exerts its tumor growth suppressor activity and terminal myeloid differentiation of granulocyte/monocyte progenitor (GMP) cells via PML-NBs in RA pathway. In addition, RA as a natural morphogen guides posterior patterning in embryo neural development. Based on these two scenarios the aim of this study was to define if there was any revenue for PML-NBs in RA dependent neural development.
Materials and Methods: For this reason, RA was used as a neural inducer for *in vitro* neural differentiation of human embryonic stem cells (hESCs). PML mRNA and protein levels were assessed by quantitative real time RT-PCR (q-RT-PCR) and western blotting in hESCs, neural precursor cells (NPCs) and mature neural cells (MNCs) obtained from this differentiation process.
Results: qRT-PCR results showed that PML had a maximum expression in hESCs and this expression decreased in NPCs and MNCs. Interestingly, three protein bands about 170, 130 and 70 kDa similarly were detected in these cell types on western blots.
Conclusion: Based on qRT-PCR results, PML expression may have an important role in cellular pluripotency. However, the appearance of three similar bands in western blots from ESCs, NPCs, and NCs led us to assumption that PML might be necessary in cellular pluripotency and nervous system development.
Keywords: Promyleocytic Lukemia Protein, Embryonic Stem Cells, Neural Precursor Cells, Neural Differentiation

Ps-76: Expression Profile of Purinergic Receptors in Human Embryonic Stem Cell and Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cells

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Objective: Embryonic stem cells (ESCs) and their derivations have significant promising roles in future cell-based therapies. In this regard, many attempts have been performed to differentiate human ESCs (hESCs) to oligodendrocyte progenitor cells (OPCs). OPCs are expected to differentiate to adult myelinating oligodendrocytes in demyelinating disease such as multiple sclerosis. Although these cells are characterized in depth based on their genetic and cell biology, there is limited information about their behavior in response to physiological or pharmacological factors. Among numerous factors that influence different aspects of their development, purinergic signaling is emerging as an important one.
Materials and Methods: This study investigated the expression level of different subtypes of purinergic receptors in hESCs and hESC-derived OPCs using quantitative RT-PCR technique to obtain further insight about developmental role of purinergic system in these cells.
Results: Our results revealed the expression of A1, A2A, A2B, and A3 adenosine receptors in hESCs. However, hESC-derived OPCs expressed all subtypes of adenosine receptors except for A2A adenosine receptor. Also, we observed several P2X (P2X2, 3, 4, 5, 7) and P2Y (P2Y1, 2, 4, 11, 12, 13, 14) mRNA expression in hESCs. hESC-derived OPCs expressed different subtype Of P2X (P2X1, 2, 3, 4, 5, 7) mRNA and P2Y (P2Y1, 2, 4, 11, 12, 13, 14) mRNA too.
Conclusion: This work is the first step to characterize the expression of purinergic receptors in hESCs and hESC-derived OPCs. More studies are necessary to evaluate the physiological role and pharmacological importance of expressed receptors in each stage of cellular differentiation.
Keywords: Human Embryonic Stem Cell, Oligodendrocyte Progenitor Cells, P1 Purinergic Receptor Subtypes, P2 Purinergic Receptor Subtype

Ps-77: Pulsed Electromagnetic Field Enhances SOX9 Gene Expression in Adipose-Derived Mesenchymal Stem Cells Encapsulated in Alginate Hydrogels

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Objective: Differentiation of mesenchymal stems cells (MSCs) by the means of chemical factors has long being understood. However, the influences of physical-electromagnetically factors are still under investigation. Further more, the culturing systems of MSCs have a significant role in determining cell fate. As alginate hydrogels support a 3 dimensional niche for cells, they are widely known as a conventional stem cell delivery systemand chondrogenesis specific gene study scaffold. SOX9 is one of the chondrogenic markers that code the SOX9 transcription factor, which plays a critical role in chondrogenesis. In the current study, we tried to assess the ability of extremely low-frequency pulsed electromagnetic field (PEMF) frequency of 25 Hz on the SOX9 gen expression potentials in adipose-derived mesenchymal stem cells (AD-MSCs) encapsulated in alginate beads.
Materials and Methods: Rabbit AD-MSCs were obtained from National Cell Bank of Iran (Pasteur Institute of Iran) cultured, at passage 4 they were harvested, and encapsulated in alginate beads. Treatments involved an 8-hour per day exposure to PEMF with the intensity of 1.6 mT, the frequency of 25 Hz, and the duty cycle of 35 ms to 5 ms for a time period of 21 days. Cellular toxicity, histological analysis, and the gene expression of SOX9 were evaluated.
Results: MTT analysis demonstrated no toxicity effect of the PEMF applied. Histological analysis showed positive alcian blue staining as a result of glycosaminoglycan synthesis. PEMF treatment resulted in a 608-fold increase in SOX9 gene expression compared to the control group. The present results suggest that PEMF with the defined parameters has the ability to increase the up-regulation of SOX9 gene expression in ADMSCs cultured inalginate constructs.
Conclusion: PEMF enhances the deposition of extracellular matrix molecules and augments SOX9 mRNA levels. Optimization of PEMF parameters can improve the chondrogenic potential of AD-MCSs and could be applied for cartilage defects.
Keywords: Adipose-Derived Mesenchymal Stem Cells, Electromagnetic Field, SOX9, Electromagnetic Transduction

Ps-78: A Comparison of The Osteogenic and Chondrogenic Potential of Mouse Dental Pulp Stem Cells

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Objective: The purpose of this study was to investigate the Chondrogenic and Osteogenic capacity of mouse dental pulp cells and identifying gene expression, cell viability, Morphological characterization for dental pulp stem cell differentiation.
Materials and Methods: For the dental stem cells culture, Alpha Modification of Eagle's Medium (AMEM), 15% FBS and 1% penicillin/streptomycin were used as a complete media. The chondrogenic medium contained ulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 ng/mL TGF- ß1, 50 µg/mL ITS+, 50 µg/mL ascorbate-2-phosphate, 100 nM Dexamethasone. For differentiate to osteoblast, 50 µg/mL ascorbic acid and 10 mM ß-glycerol phosphate and to induce osteoclast differentiation, 10 ng/mL RANKL and 5 ng/mL M-CSF were added to complete medium.
Results: In RT-PCR molecular analysis, mouse dental pulp stem cells (DPSC) were observed to express Cd146 and Cd166 genes, which indicated that these cells belong to mesenchymal stem cells. Collagen II as mature chondrocyte markers and OPN as an osteoblast marker were expressed after 14 days and 21 days during chondrogenic and osteoblastic differentiation, respectively. While the non activation of CatK as osteoclast markers after induction, indicates these cells have not differentiated into osteoclast. After 21 days the cell morphological characters were changed when exposed to chondrogenic and osteoblastic induction medium whereas similar morphological were produced in both control and osteoclast differentiated medium groups. Using toluidine blue staining and von Kossa staining indicated the presence of glycosaminoglycans and calcium nodules which is evident of successful chondrogenic and osteoblastic differentiation, respectively. Cell viability during the differentiation showed that these cells preserved their viability during differentiation, however after 16 days and 7 days in osteoblast and chondrocyte differentiation medium respectively, the amount of differentiated cells viability as compared to the control cells was significantly decreased ($p<0.05$).
Conclusion: This study indicated that DPSC with high proliferation rate had chondrogenic and osteoblastic differentiation capacity but does not possess osteoclastic differentiation ability.
Keywords: Dental Pulp Stem Cell, Osteogenic, Chondrogenic

Ps-79: Laminin Coating Stimulates Polarity, Protein Synthesis, and Metabolism of Hepatocyte-Like Cells Differentiated from Human BM-MSCs

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Objective: Application of stem cells in cell therapy and tissue engineering for treatment of liver diseases

are considered as alternative approach to liver transplantation. So far, extensive studies have been taken to improve hepatogenic differentiation efficiency of different types of stem cells; but not much of interest has been paid to the polarity of the *in vitro* differentiated cells. The aim of our study was to investigate the effect of laminin on polarity of hepatocyte-like cells differentiated from Human bone marrow (BM)-derived mesenchymal stem cells (BM-MSCs) as well as the protein synthesis and metabolism.

Materials and Methods: The characteristics of MSCs obtained from Royan Institute were confirmed by immunophenotyping analysis and their differentiation potential into osteocytes and adipocytes. Hepatogenic differentiation was induced by hepatocyte growth factor, Oncostatin M, and dexamethasone. Undifferentiated human MSCs were used as negative control. On day 21, Immunostaining for albumin (Alb) and alpha-fetoprotein (AFP) were performed to evaluate the characteristics of the differentiated cells. Furthermore, urea production was evaluated to measure the detoxification capacity of the cells. Finally, F-actin staining was performed to analyze the polarity.

Results: The flowcytometry findings revealed that over 90% of the cells expressed the MSC markers but not hematopoietic and leukocyte markers. Also mineralization and oil droplets showed their differentiation potential into osteocytes and adipocytes. The results of immunocytochemistry revealed higher expressions of both AFP and Alb (specific markers of liver) in cells differentiated on laminin matrix compared to that of the control group. Additionally, the results showed that urea production by cells differentiated on laminin was significantly more than that of the polystyrene. Furthermore, few bile canaliculi structures were detected by F-actin staining, in the cells differentiated on polystyrene, while in cells differentiated on laminin the number of detected bile canaliculi was higher.

Conclusion: Taken together, these findings may indicate that laminin coating can improve the terminal differentiation of hepatocyte-like cells from human BM-MSCs, by stimulating their protein synthesis, metabolism, and polarity which is fundamental for their function. Thus, laminin might be considered as a suitable coating in hepatic tissue engineering designs.

Keywords: Differentiation, Hepatocyte, Laminin, Mesenchymal Stem Cell, Polarity

Ps-80: Hepatic Differentiation Potential of Menstrual Blood- versus Bone Marrow-Stem Cells

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Objective: Menstrual blood has been introduced as a simply accessible source for stem cell. The aim of this study was to investigate hepatic differentiation capacity of menstrual blood stem cells (MenSCs) compared to bone marrow-derived stem cells (BMSCs) under different protocols.

Materials and Methods: Menstrual blood was collected from healthy females using Diva cup. Bone marrow aspirates were obtained from iliac crests of human donors. Samples were isolated using a combination of density gradient centrifugation and plastic adherence. Differentiation was induced by treating cells with different concentrations of hepatocyte growth factor (HGF) and oncostatin M (OSM) in combination with other components in culture media (P1, P2, and P3 protocols). Parallel experiments were carried out to assess expression of hepatic markers at mRNA and protein levels. Furthermore, Glycogen storage and albumin (ALB) secretion were evaluated to find out functionality of differentiated cells.

Results: The differential expression of mature hepatocyte markers such as albumin (ALB), cytokeratin 18 (CK-18), tyrosine aminotransferase and cholesterol 7 alpha-hydroxylase activities (CYP7A1) at both mRNA and protein levels in differentiating MenSCs was significantly higher in upper concentration of HGF and OSM (P1) compared to lower concentration of these factors (P2). Moreover, omission of serum during differentiation process (P3) caused typical improvement in functions assigned to hepatocytes in differentiated MenSCs. While up-regulation level of ALB and CYP7A1 was higher in differentiated MenSCs compared to driven BMSCs, expression level of CK-18, detected level of produced ALB and glycogen accumulation were lower or not significantly different.

Conclusion: We proved MenSCs have capability to generate functional hepatocyte-like cells, although they showed different expression pattern compared to BMSCs depend on critical growth factor concentration and culture media condition.

Keywords: Menstrual Blood Stem Cells, Differentiation, Hepatocyte, Stem Cell

Ps-81: Three Dimensional Culturing System and Fibroblast Growth Factor-4 Improved Expression of Hepatic Nuclear Factor-4 as A Main Gene in Hepatogenesis

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Objective: The aim of this study was to find the ef-

fect of 3D culture system and FGF-4 on the expression of HNF-4 by HWJMSC which is differentiated toward hepatocyte. The scaffolds and the growth factors are considered as a cell culture support that impact stem cell differentiation. FGF4 is a bioactive protein that has mitogenic activity and induces the cells toward definitive endoderm. HNF 4 has a significant role in the differentiation of the hepatocyte lineage. It was shown that HNF4 secreted from the visceral endoderm and required for the expression of several factors in the liver-specific phenotype through induction of various liver-specific functions

Materials and Methods: MSCs derived from Wharton's jelly explants were characterized by flow cytometry. Then, the cells were cultured in the presence of hepatogenic media with or without FGF4 on 2D and 3D collagen scaffold for 21 days. The expression of HNF-4 was evaluated by Real time RT-PCR.

Results: The cultures pre-exposed to FGF4 in 3D culture system expressed higher levels of HNF-4, compared to the other cultures.

Conclusion: The result showed that both FGF-4 and 3D culture system influence on over expression of HNF-4. 3D culture system mimicked the intracellular microenvironment and improved the hepatic differentiation of human MSCs derived from Wharton's jelly.

Keywords: FGF-4, HWJMSC, HNF-4, 3D Culture System, Hepatocyte

Ps-82: NKX2-5eGFP/w hES Cell Model for Studying Mitochondrial Cardiomyopathy

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Objective: Assembly and function of mitochondrial respiratory chain complexes rely on proteins encoded by hundreds of genes. Mutations in any of these mtDNA or nuclear genes can cause dysfunction of any cell type. Higher energy demand of tissues like skeletal or cardiac muscle and the central nervous system are frequently affected by mitochondrial disorders. Patients with mitochondrial cardiomyopathy often have normal function of the respiratory chain in cell lines like skin fibroblasts that are easily obtained. Studying the mechanism of organ dysfunction without a good model system is thus difficult. To overcome this issue we generated a novel cellular model system to study mitochondrial disorders.

Materials and Methods: NKX2-5eGFP/w cells are human Embryonic Stem Cells (ESCs) in which eGFP expression is controlled by the NKX2-5 locus to enable quantification of cardiac differentiation and purification of cardiac progenitor cells (hESC-CPCs) and cardiomyocytes (hESC-CMs). RNA-guided DNA cleavage technology was used for genome editing to knock out genes involved in mitochondrial cardiomyopathy in this cell line. Cells were FACS sorted and single cell cultured for colony formation and further characterization. Mutated cells were confirmed by testing in different culture conditions.

Results: The system represents a promising approach for targeted knock-out of genes in NKX2-5eGFP/w hESCs. These hESCs can then be differentiated to functional cardiomyocytes to study the cell-specific mechanisms of mitochondrial cardiomyopathy.

Conclusion: hESCs which carry a mitochondrial respiratory chain defect and are differentiated to functional cardiac cells provide an excellent model system. They can be used for validation of novel mitochondrial disease genes in which mutations cause cardiac-specific pathology that cannot be studied in cells or tissues that are normally available. This model system provides a way to explore tissue-specific genetic diseases like heart failure and cardiomyopathy in newborns.

Keywords: Mitochondrial Cardiomyopathy, Differentiated Cardiomyocytes, Mitochondrial Disorder, RNA-Guided Genome Editing

Ps-83: Mutations Detection in Patients with Wolfram Syndrome from Iran

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Objective: Wolfram Syndrome is a rare genetic disorder inherited in an autosomal recessive mode. It is considered as a neurodegenerative disorder also known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness). Mutations in WFS1 gene are found to be associated with the disease. WFS1 is located on chromosome 4p16.1 and consists of 8 exons. Wolfram Syndrome is mostly caused by mutations of exon 8 of this gene. In this study we investigated mutations in exon 8 of WSF1 gene which consists of 7 fragments in Iranian patients.

Materials and Methods: Four male patients were coded as WF023, WF024, WF029, WF030 were investigated and their clinical symptoms were compatible with Wolfarm syndrome. DNA was extracted from peripheral blood of each patient and exon 8 of WFS1 gene was amplified by PCR method and direct sequencing was used for mutation detection in 7 fragments of this exon (8a-8g).

Results: We found V333I mutation in fragment 8a of all patients. A novel mutation (G736D) was also

found in fragment 8e of case WF023. Fragment 8a of patients WF029 and WF030 showed rs367605880 polymorphism. Other polymorphisms, rs56072215 in fragment 8a, rs2230719 in fragment 8c, rs2230721 and rs1046316 in fragment 8f of case WF030 were detected. In case WF030 we have also found three 3'UTR variations (rs1046317, rs1046319, rs1802453) in fragment 8g.

Conclusion: The V333I mutation that we found in fragment 8a of all cases has also been reported in previous studies. However in some studies this was reported as a polymorphism. The novel G736D has been first time observed in patients with Wolfram syndrome in our study. This mutation was reported in 1000 genome database. Other polymorphisms found in our study have also been reported in previous investigations. Overall, it seems that more investigations is needed to exactly identify mutations related to Wolfram Syndrome in Iranian population.

Keywords: Wolfram Syndrome, WFS1 Gene, Iran

Ps-84: MicroRNA Expression Profile of Colon Cancer Stem Cells Compared to Non-Stem Cells

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Objective: Cancer stem cells (CSC) have been reported in many human tumors and are thought to be responsible for tumor initiation, therapy resistance, progression, relapse, and metastasis. MicroRNAs (miRNAs) play important roles in maintaining stemness of embryonic stem cells and CSCs. So, we evaluated the miRNA expression profile in primary colon cancer stem cells compared to non-stem cells to determine the relationship between tumor stem cells and tumor biological behavior.

Materials and Methods: Primary colon cancer cells were cultured in serum free medium to form colonospheres. colon CSCs biomarkers including CD44 and EPCAM as well as expression levels of stemness genes in colonospheres and parent cells were investigated. In addition, in vivo xenografts assay was performed to determine tumorigenic potential of tumor spheroid cells. Moreover, we evaluated the miRNA expression profile of colon CSCs using miRNA PCR array.

Results: The spheroid cells with high expression of EPCAM/CD44 markers showed higher tumorigenic potential than parental cells ($p<0.05$). Furthermore, expression of stemness genes were higher in spheroid cells compared to parental cells ($p<0.05$). There are 39 differentially expressed miRNAs, 24 of them had lower mean expression in the cancer stem cell samples. Conversely, 15 miRNAs had higher expression levels in CSCs samples. Of these, miR-495, miR-125, miR-199a were the most significantly up-regulated miRNAs.

Conclusion: Our results suggest that miRNAs might play important roles in maintenance and regulation of colon CSCs and specific miRNA expression signatures may contribute to cancer initiation and expansion.

Keywords: Colorectal Cancer, Cancer Stem Cells, MicroRNA

Ps-85: ALDH1: Is A Useful Biomarker for Identifying Colon Cancer Stem-Like Cells in HT-29?

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Objective: Recent evidence suggested that epithelial cancers including colorectal cancer (CRC) are driven by a small population of self-renewing, multi-potent cells termed cancer stem cells (CSCs) which could be responsible for the recurrence of cancer. Aldehyde dehydrogenase 1 (ALDH1) activity has been used as a functional stem cell biomarker to isolate CSCs in different cancers such as colorectal cancer. The main aim of this research was to determine the utility of ALDH1 activity along with CD44 and EPCAM in identifying stem cell–like cells in human HT-29 colonic adenocarcinoma cell line.

Materials and Methods: In the present study, colon CSCs biomarkers including CD44, EPCAM and ALDH1 were analyzed by flow cytometry in colonospheres and parent cells. The expression levels of stemness genes in spheroid and parental cells were investigated using real-time PCR. In addition, in vivo xenografts assay was performed to determine tumorigenic potential of tumor spheroid cells.

Results: According to the results, over 96% of spheroids were CD44+/EpCAM+, while only 37% expressed CD44/EpCAM biomarkers. Controversially, ALDH activity was about 2-fold higher in the parent cells than inspheroid cells. Compared with the parental cells, expression levels of ''stemness'' genes, like Sox2, Oct4, Nanog, C-myc, and Klf4 were significantly increased in colonosphere cells ($p < 0.05$). Further, administration of 2500 spheroids could be sufficient to

initiate tumor growth in nude mice, while 1x106 of parental cells was needed to form a tumor.
Conclusion: It doesn't seem that ALDH1 would be a useful biomarker to identify CSCs population in HT-29 cell line. However, colonospheres with low ALDH1 activity indicated increased tumorigenic potential and stemness properties.
Keywords: Colorectal Cancer, Cancer Stem Cell, HT-29, ALDH, Biomarker

Ps-86: Identification and Characterization of Cancer Stem Cells in Colon Adenocarcinoma Patients

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Objective: Proliferation and expansion of cancer stem cells as spheroids were proved in previous studies. But, capability of primary tumor derived cancer stem cells to keep their special properties *in vitro* is still disputed. So, the goal of this study is to isolate, expand and characterize primary tumor derived colon cancer stem cells
Materials and Methods: In the present work, colon CSCs markers including CD44 and EPCAM in spheroid and parental cells were analyzed by flow cytometry. The expression levels of stemness genes in both spheroid and parental cells were investigated using real-time PCR. Tumorigenic potential of spheroid cells was evaluated using in vivo xenografts assay
Results: Our data showed 79% of spheroids were CD44+/EpCAM+, while only 20% of parental cells expressed CD44/EpCAM markers. In compared with the parental cells, expression levels of ''stemness'' genes, like Sox2, Oct4, Nanog, C-myc, and Klf4 were significantly increased in spheroid cells ($p< 0.05$). Further, As little as 1000 spheroid cells were sufficient to obtain tumor growth in nude mice, while $1x10^6$ of parental cells was needed to form tumor.
Conclusion: Sphere formation assay is a useful method for enrich cancer stem cells. Spheroid cells showed increasing expression of stemness genes and tumorigenesis in nude mice.
Keywords: Cancer Stem Cells, Colon Cancer, Spheroid, CD44, EPCAM

Ps-87: A Novel, Safe Strategy to Derive Naïve-Like Human Pluripotent Stem Cells

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Objective: Although human and mouse embryonic stem cells (ESCs) are both derived from the inner cell mass of the blastocysts, they exhibit distinct characteristics particularly in terms of developmental potential. While mouse ESCs exist in a naïve pluripotent state, human ESCs are in a primed state, displaying a propensity to better differentiate into certain cell types than others. Induction of naïve pluripotency in the human pluripotent stem cells (PSCs) is thus of particular importance to obtain pluripotent cells with less differentiation propensity. Here we report a novel safe approach to derive naïve-like human PSCs.
Materials and Methods: Primed human ESCs and induced pluripotent stem cells (iPSCs) were first cultured in a mouse ESC culture medium containing LIF and the 2i inhibitors: PD0325901 which inhibits FGF signaling and CHIR99021 which blocks the GSK3 pathway. Next the cells were treated for five to seven days with specific small molecules. Once emerged, the naïve-like colonies were trypsinized and plated onto feeder-coated plates for expansion. Finally, naïve-like cells were characterized for properties of naïve pluripotency.
Results: Our small-molecule cocktail could successfully induce naïve-like human PSCs which exhibited the naïve characteristics in terms of morphology, expandability, doubling time, single cell survival, single cell clonogenicity, LIF responsiveness, XIST repression, *in vitro* and in vivo differentiation, enhanced NANOG and E-Cadherin expression, and nuclear localization of the proteins STAT3 and TFE3.
Conclusion: We have successfully devised a novel culture system for the safe derivation of naïve-like human PSCs from primed human PSCs using the combination of two chemicals under the 2i+LIF culture condition.
Keywords: Pluripotency, Naïve State, Primed State, Small Molecule

Ps-88: The Role of Mir-129-1 Upregulation in The Growth and Proliferation of Glioblastoma Multiforme Cancer Cells

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Objective: Glioblastoma multiforme (GBM) is a complex heterogenic disease and microRNAs (miRNA) through their specific targets could impact on various aspects of GBM heterogeneity including tumor proliferation. So, in the peresent study, we investigated the functional role of miR-129-1 in gliomagenesis.
Materials and Methods: Three GBM cell lines was infected with lentiviruses containing mir-129-1 precursor sequence. The effects of ectopic expression of miR-129-1 on GBM phenotype were examined by cell cycle analysis and apoptotic assays and MTT.
Results: Restoration of miR-129-1 in GBM cell lines dramatically reduced cell growth and induced G1 arrest. Using a combined bioinformatics and molecular approach, we recognized two novel putative targets of miR-129-1 involved in GBM pathogenesis.
Conclusion: Our data suggest a tumor suppressor role for miR-129-1 and reveal that the up-regulation of miR-129-1 would be a potential approach for GBM cancer therapy that induces G1 arrest.
Keywords: MicroRNA-129-1, GBM Cancer, Cell Cycle Arrest

Ps-89: Generation of Porcine Induced Pluripotent Stem Cells from mCherry Expressing Fibroblasts Mediated through PiggyBac Transposon

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Objective: Despite advances in understanding of human Induced Pluripotent Stem (iPS) cells, their safety for potential cell therapies has to be proved in appropriate animal models. A limitation of murine models for preclinical assessments of innovative cell therapies is the short life span, small size, and the high level of inbreeding in this species. The pig is an attractive large animal model for preclinical testing of safety and efficacy of cell based therapies. Porcine organs are largely similar in size and physiology to their human counterparts rendering the domestic pig a suitable model for cardiovascular disease, muscular dystrophies, atherosclerosis, wound repair, diabetes and ophthalmological diseases. Therefore, the present study was carried out to derive porcine iPS cells from transgenic fetuses systemically expressing mCherry through a non-viral piggyBac transposon. Flurophore (mCherry)-labelled porcine iPS cells may turn out as important tool for blastocyst complementation assays.
Materials and Methods: Porcine fetal fibroblasts isolated from mCherry porcine fetuses at passage 2 described by Garrels et al., 2011, were co-electroporated with a PB transposon carrying a multigene cassette consisting of the cDNAs for Oct4, Sox2, Klf4, cMyc, Nanog, and Lin28 separated by self-cleaving 2A peptide sequences and a helper plasmid expressing the pCMV-PB transposase. On day 6 post electroporation morphology of fibroblasts started change to round structure and on day 9 cell colonies appeared. These putative iPS cells were cultured, propagated and characterized as described by.
Results: In this study, the morphology of generated iPS cells was similar to ES cells and they were clonally propagated up to passage 30 and then cryopreserved for future used. The iPS cells were characterized through immunostaining and found positive for AP, OCT4, NANOG, SOX-2, SSEA-1 and SSEA-4. Expression of stemness genes OCT4, SOX-2, NANOG, c-MYC, KLF, UTF, E-CADHERIN, CHD, STELLA, TDH and GAPDH was detected by RT-PCR. *In vitro* differentiation potential was assessed by Embryoid Bodies (EBs) formation. The formed EBs exhibited the expression of mCherry in their cells and expressed genes like NESTIN, TUJI, GATA4 and AFP. To test their tumorigenic potential, 1×10^6 porcine iPS cells were injected under the skin of immune deficient nude mice. A visible tumor growth was observed 6 weeks later.
Conclusion: This study indicates that piggyBac transposon containing six transcription factors is able to reprogram the porcine fetal fibroblast into iPS cells. These cells could be cultured and maintained *in vitro* for a prolonged period, exhibit characteristics of stem cells and offer a potential source for future blastocyst complementation experiments.
Keywords: iPS Cells, Pluripotency Markers, Fibroblasts, Teratoma, Differentiation

Ps-90: Heat Shock Instructs HESCs to Exit from The Self Renewal Program through Negative Regulation of OCT4 by SAPK/JNK and HSF1 Pathway

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Objective: Environmental factors affect self-renewal of stem cells by modulating the components of self-renewal networks. Heat shock, an environmental factor, induces heat shock factors (HSFs), which up-regulate stress response-related genes. However, the link of heat shock to self-renewal of stem cells has not been elucidated yet. Here, we present the direct link of heat shock to a core stem cell regulator, OCT4, in the self-renewal network through SAPK/JNK and HSF1 pathway.
Materials and Methods and Results: We first

showed that heat shock initiated differentiation of human embryonic stem cells (hESCs). Gene expression analysis revealed that heat shock increased the expression of many genes involved in cellular processes related to differentiation of stem cells. We then examined the effects of HSFs induced by heat shock on core self-renewal factors. Among HSFs, heat shock induced mainly HSF1 in hESCs. The HSF1 repressed the expression of OCT4, leading to the differentiation of hESCs and the above differentiation-related gene expression change. We further examined the effects of the upstream mitogen-activated protein (MAP) kinases of HSF1 on the repression of OCT4 expression by HSF1. Among the MAP kinases, SAPK/JNK controlled predominantly the repression of the OCT4 expression by HSF1.

Conclusion: The direct link of heat shock to the core self-renewal regulator through SAPK/JNK and HSF1 provides a fundamental basis for understanding the effect of heat and other stresses. This involves activation of HSF1 on the self-renewal program and further controlling differentiation of hESCs in a broad spectrum of stem cell applications using these stresses.

Keywords: Heat Shock, HSF, MAPK, OCT4, Pluripotency, hESCs

Ps-91: ES Cell-Specific miR-302 Reprograms Skin and Colon Cancer Cells, and Modulates Apoptosis, Metastasis and Angiogenesis Markers

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Objective: The miR-302 cluster is the most abundantly expressed miRNA in human embryonic stem cells which is subsequently down-regulated during the process of differentiation. Previous studies have shown that transfection of some cancer cell lines with miR-302 expressing vectors can reprogram these cells and transform them into an embryonic stem-like state with a lower proliferation rate.

Materials and Methods: In the current study, melanoma A-375 and colorectal adenocarcinoma HT-29 cancer cell lines were transfected with pEGFPC1-miR-302a/b/c/d and mock vectors. The expression of some pluripotency, apoptotic, invasion, and angiogenesis genes was evaluated by RT-PCR and quantitative real-time PCR. Furthermore, the expression of Oct4 and Sox2 proteins was assessed by immunocytochemistry. Cell cycle analysis was performed by propidium iodide (PI) flowcytometric assay.

Results: The pluripotency markers were upregulated after transfection with miR-302 cluster. According to the cell cycle analysis, a significant proportion of the A-375-miR-302 cells were arrested in G2 phase, and apoptosis was induced in about eight percent of the cells. Moreover, the expression of some apoptotic genes was increased in A-375 cell line. There was no significant cell death after transfection of HT-29 cell line with miR-302 cluster while the proliferation rate was mildly decreased. Additionally, the expression of some apoptotic and cell cycle regulator genes in HT-29 cell line was upregulated following miR-302 transfection. There was also an upregulation of CDH1 and downregulation of SNAIL, HIF1A, and VEGFA in both cell lines. However, the expression levels of MMP2, HIF1B, and DLL4 were decreased exclusively in the A-375 cell line. Increased expression of MMP2 and DLL4 genes in HT-29 cell line was one of the most controversial observations in this study.

Conclusion: Results of this study revealed that transfection of A-375 and HT-29 cancer cells by miR-302 cluster can upregulate the expression of pluripotency genes in both cell lines. Moreover, this cluster can induce cell cycle arrest and apoptosis primarily in the melanoma cell line. Furthermore, overexpression of miR-302 cluster may disrupt various signaling pathways which are involved in different stages of metastasis and angiogenesis through modulation of particular target mRNAs.

Keywords: Reprogramming, miR-302, Apoptosis, Pluripotency, Metastasis

Ps-92: Assessment of Mvh Expression Following Treatment with Phoenix Dactylifera Date Palm Pollen in Neonate Mouse Spermatogonial Colony

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Objective: Phoenix dactylifera date palm pollen (DPP) is one of the traditional medicines for male infertility treatment. This study aimed to evaluate the *in vitro* effects of aqueous extract of DPP on the Mvh (The germ cell specific gene) expression upon neonate mouse spermatogonial stem cells (SSCs) colony formation.

Materials and Methods: Cell suspension includes sertoli cells and SSCs were isolated from neonatal 6 day-old mice testes by 2 steps enzymatic digestion and cultured in a DMEM and FCS 4% in the absence or presence of different doses of aqueous extract of DPP (0.06, 0.25 and 0.62 mg/ml) for 2 weeks. The expression of Mvh (VASA) was assessed using Real Time PCR at the end of the culture period. Statistical analysis was determined using ANOVA and paired samples t test at the level of $p \leq 0.05$.

Results: After 2 weeks of culture, there were no significant differences in the expression of Mvh gene between control (0.000064 ± 0.00008), 0.06 mg/ml

(5.11 ± 6.86), 0.25 mg/ml (0.0157 ± 0.022) and 0.62 mg/ml concentration (0.000082 ± 0.00004) groups.
Conclusion: Our results showed that in the colonies derived from SSCs, the expression of Mvh gene were not significantly increased in the presence of different doses of aqueous extract of DPP.
Keywords: Spermatogonial Stem Cell, Palm Pollen

Ps-93: The Effect of Aqueous Extract of Phoenix Dactylifera Pollen on *In Vitro* Viability and Proliferation Rate of Neonatal Mouse Spermatogonial Stem Cells

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Objective: There is a fast growing tendency in the consumption of herbal remedies in the developing countries. One of the traditional medicines used for male infertility is Date palm (Phoenix dactylifera) pollen (DPP). The goal of the present study was to investigate the effect of aqueous extract of DPP on *In vitro* viability and proliferation rate of neonate mouse spermatogonial stem cells (SSCs).
Materials and Methods: cell suspension includes sertoli cells and SSCs were isolated from neonatal 6 day-old mice testes by 2 steps enzymatic digestion. The cell suspension was cultured in DMEM and FCS 4% in the absence or presence of 0.06, 0.25 and 0.62 mg/ml of aqueous extract of DPP for 2 weeks. In order to evaluate the rate of SSCs expansion at the end of culture, the mean number of whole cells and living cells were considered as proliferation and survival rates respectively.
Results: The results showed that there were no significant differences between the mean percent of viability and proliferation rate between control and 0.06, 0.25 and 0.62 mg/ml of DPP-treated groups ($p>0.05$).
Conclusion: Our study showed that treatment of neonatal mouse testicular cell suspension with DPP had no toxic effects on viability percent and proliferation rate of these cells. Thus, we can use DPP for evaluating the *in vitro* pattern of SSCs colonization in the future studies.
Keywords: Date Palm Pollen, Viability, Proliferation

Ps-94: Effect of Umbilical Cord Blood Derived Platelet-Lysate on Fibroblast Proliferation and Activation of Smad Signaling Pathway

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Objective: Bioactive substances of platelets play a pivotal role in wound healing. To investigate the mechanisms underlying platelet-induced wound healing using fibroblast, we studied the effect of umbilical cord blood platelet lysate (UCB-PL) on fibroblast, as potent cell in wound healing. In addition, the effect of UCB-PL on the activation of smad 2/3 after wound scratching have been studied.
Materials and Methods: We prepared platelet extract from cord blood plasma, then (1×10^5 cells/ml) fibroblast was treated by different concentrations of UCB-PL (5-8-10-15-20%) the mixture of UCB-PL and fetal bovine serum for 24, 48, and 72 hours. Cell viability and proliferation rate were assessed using manually count by trypan blue. Scratch assay was used to evaluate wound healing *in vitro* were assessed using manually count by trypan blue scratch assay respectively. Then the expression of gene smad2- smad3 was determined by real-time PCR .Then we used western blotting for analysis in order to investigate psmad2/3, smad2/3 signaling pathway.
Results: Our results demonstrate an increase in cell proliferation at concentration of 10% of UCB-PL without any effect on cell viability. The same dose of UCB-PL induced a significant increase of wound closure rate at 6, 12 and 24 hours after treatment. However it was observed that the increased gene expression ratio in smad3 and smad2 genes at 10% of UCB-PL compare with negative and positive controls significant effect on protein levels on smad3, smad2, psmad2 and psmad3 proteins.
Conclusion: UCB-PL can be a satiable supplement for fibroblast culture *in vitro* and stimulates fibroblast growth and motility which induce wound healing through the activation of different signaling pathways including smad 2/3 signaling pathway in vivo.
Keywords: Umbilical Cord Blood, Wound Healing, Platelet-lysate, Smad 2/3 Signaling Pathway

Ps-95: Empirical Studies on The Impact of the Application of Vitamin A and C on The Capabalities of The Scaffold Made from Fish Swimming Bag in The presence of Blastema Cells of Rabbit's Ear *In vitro*

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Objective: In recent decades regenerative medicine have been widely developed, which in turn led to the

most important advances in biomedical research as well as clinical efforts. One of the new challenges is to explore concepts of construction and repair of human tissues by the use of living biological materials through universalizing engineering technologies. In the current study, the use of vitamin A and C in optimizing decellularized scaffold of fish swimming bag has been studied in order to develop the capabilities of scaffolds, including the possibility of a blastema cell adhesion, motility and cell differentiation.

Materials and Methods: At first the salmon swim bag was decellularized by the use of Sodium Dodecyl Sulfate. Scaffold impregnation with vitamin A and C was conducted to increase the capabalities of scaffold. Then the tissue and blastema cells in rabbit ear were assembled in decellularized scaffold. At weeks 1, 2 and 4 histologic and histochemichal studies were done in the absence and presence of vitamin A and C to study cell behaviors including motility, proliferation and chondrogenic differentiation or morphological changes of blastema cells.

Results: It seems that the strength and vitality of natural decellularized scaffold will increase in the presence of vitamines. And the presence of these compounds will have a positive impact on the morphology and behaviors of the blastema cells including adhesion to the matrix,migration induction,proliferation and differentiation.

Conclusion: Efforts to increase the application of biocompatible structures in order to conduct preliminary studies of tissue engineering and studies of the interaction between living cells and ECM scaffold and their impact on cell behavior, may lead into producing and promoting ideal natural scaffolds; or If damaged sites are treated with proper biocompatible bio-active agents such as growth factors or vitamins, which stimulate repairing functional tissue like normal positions, it can be influential in optimizing biological scaffolds. However, performing other special techniques are thought as necessary to understand more accurate mechanisms.

Keywords: Regenerative Medicine, Natural Scaffold, Vitamin A and C, Extracellular Matrix of Fish Swimming Bag, Blastema Cell

Ps-96: Investigation of Effect Condition Medium Derived from Cultured Pancreas on Differentiation of Insulin Secreting Cells from Stem Cells

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Objective: About 150 millions people are affected to diabetes mellitus through the world. Recent studies have shown that many types of stem cells can be possible sources to derive transplantable insulin-producing cells (IPCs). The aim of the present study was to investigate the effects of pancreas conditioned medium on production of IPCs from undifferentiated Embryonal carcinoma cells (EC).

Materials and Methods: In this study, the c.m of cultured pancreas from one-week newborn mouse was used for differentiation of P19. EBs formed by a 24 hour suspension culture of P19 EC cells. In order to induce the difrentiation, different concentrations of c.m (25, 50, 75 and 100%) were added to culture medium. Dithizone staining was used for the detection of EC-derived IPCs. Insulinproinsulin production and insulin receptor beta were determined by immunofluorescence. The expression of pdx-1 gene was also analyzed by reverse transcriptase-polymerase chain reaction. Data were analyzed using one way ANOVA and Dunkana test.

Results: Differentiated cell clusters appeared after approximately 7 days induction. The peak response of differentiation was at the concentration of 50% c.m. The differentiated cells were immunoreactive for β cell-specific proteins, insulin-proinsulin and β receptor. RT-PCR showed the expression of a pancreatic β cell-related gene, Pdx-1. The data presented showed that it is possible to generate IPCs from undifferentiated EC cells with the characteristics of pancreatic β cells.

Conclusion: A major obstacle in Pancreatic islet transplantation is the inadequacy of pancreatic islets. Many studies have done to expand pancreatic islets and to develop renewable sources of islet-replacement tissue. EC stem cells are developmentaly pluripotent cells which can be differentiated into all cell types under the appropriate conditions.

Keywords: Embryonic Carcinoma Stem Cells, Condition Medium, Insulin-proinsulin, Beta Receptor, Pdx-1

Ps-97: Detection of Mycoplasma Contamination in Hair Follicle Stem Cells Culture

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Objective: Stem cells such as hair follicle stem cells are important for research and cell therapy. Nestin-expressing stem cells have been previously shown to be pluripotent and be able to form neurons and other non-follicle cell types. One of the aims of this study was to detect frequent mycoplasma species in hair follicle stem cells culture (M. hyorhinis and M. arginini) with

culture and PCR methods.

Materials and Methods: In this study young mouse hair follicle, human fetal shoan stem cells, Hela and vero cell lines were used for detection of mycoplasma. First, the stem cells and cell lines were cultured for 14 days after thawing, and supernatant of these cultures were taken after a culture period of at least 5 days. Then, supernatant of these cell cultures were used for samples and were divided into 2 sections: first section was passed through 0.45 μm pore-size filters, then inoculated into specific PPLO broth and agar media and incubated at 37°C under 5% CO_2 in 7 days . Culture method were only used for detection of M. arginini because M. hyorhinis is non-cultivable. The second section was used for PCR in which primers were used for amplification of 16S rRNA gene of M. hyorhinis and M. arginini.

Results: From a total of 8 samples tested, 0% were positive with culture and with PCR method for M. arginini and also 0% were positive with PCR method for M. hyorhinis.

Conclusion: Results of this study showed that no contamination in cell lines especially in stem cells with culture and PCR methods. Since good practice in the laboratory and the frequent monitoring of the stem cell lines is important for this research.

Keywords: Contamination, Mycoplasma, Hair Follicle Stem Cells

Ps-98: Liver Fibrosis Treatment Using Secreta of Bone Marrow Mesenchymal Stem Cells in Rat

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Objective: Mesenchymal stem cells therapy may prevent parenchymal cell loss and promotes tissue repair through the action of trophic secreted molecules. This study evaluated the hypothesis that infusions of secreta (conditioned culture medium) of bone marrow mesenchymal stem cell can improve liver fibrosis in an experimental rat model.

Materials and Methods: Eighteen rats with induced liver fibrosis using thioacetamide (30 intraperitoneal injections biweekly) were divided into three groups. Two groups were intraperitoneally injected with secreta of bone marrow mesenchymal stem cells (1 ml of culture medium of 90% cell confidence without cell) 20 days after the last thioacetamide injection. They were euthanized 4 and 6 weeks after transplantation. The last group was selected as control and was euthanized 20 days after the last thioacetamide injection. Liver samples were histopathologically evaluated using Hematoxylin and Eosin staining and Masson's trichrome staining. Serum alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and albumin concentrations were compared between groups. Serum parameters were evaluated using one-way ANOVA. Histopathologic scores were analyzed using Mann-Whitney test (SPSS 11.5). $P<0.05$ was considered significant.

Results: Injection of secreta of bone marrow mesenchymal stem cells improved survival, liver fibrosis and necrosis in rats with thioacetamide-induced liver fibrosis. Secreta of bone marrow-derived mesenchymal stem cells also significantly recovered serum concentrations of alanine aminotransferase after 4 and 6 weeks (93.83 ± 3.42 U/L and 73.40 ± 2.65 U/L, respectively) and alkaline phosphatase after 6 weeks (281.80 ± 39.53 U/L) in comparison with control (132.0 ± 17.95 U/L and 488.33 ± 89.05 U/L, respectively).

Conclusion: Bone marrow mesenchymal stem cell secreta exosomes can relieve thioacetamide-induced liver fibrosis. This provides a novel approach for the treatment of fibrotic liver disease.

Keywords: Secretion, Bone Marrow, Mesenchymal Stem Cell, Liver, Fibrosis

Ps-99: The Effect of Substrate Elasticity on Cell Adhesion Force of Endothelial Cells

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Objective: Cells have dynamic structures with various functions, such as adhesion, migration, and signal transduction which can be dramatically influenced by their extracellular environment. Cells respond to their environmental cues including chemical, topological, and mechanical signals to maintain homeostasis for their function as well as their integrity. The cellular responses are in accordance with stimuli transmitted by adhesion protein receptors which create intracellular signaling resulting in stronger adhesions of the contact cell or cell motility. Many pathological diseases can be caused by abnormal cellular or tissue mechanics and mechanotransduction caused by environmental change.

Materials and Methods: In this study, we used Poly acryl amid (PAA) to create three substrates with different stiffness in order to see the effect of substrate elasticity on cell adhesion force. Human umbilical vein endothelial cells (HUVEC) obtained from National Cell Bank of Iran (Pasteur Institute of Iran) were used to determine the toxicity of substrates using the extracts through MTT assay. Surface topology and elastic moduli of substrates were assessed by means of Atomic

Force Microscopy (AFM). Cell-substrate adhesion force of endothelial cells on different substrates was estimated using Single Cell Force Spectroscopy (SCFS).
Results: MTT results showed more than 90% cell viability for all three samples. Endothelial cells were attached more tightly to the substrate with the highest stiffness while cells are more spherical-like on the softest substrate. Measured cell-substrate adhesion force demonstrated a significant increase from a soft substrate to a hard one that is 328, 418.8 and 783 nN on soft, medium, and hard substrates respectively. The results of topography depicted the similar topography of three different surfaces.
Conclusion: The results of this study revealed that cell adhesion is actively responding to any changes in the mechanical properties of its extracellular matrix. These findings emphasize the importance of substrate stiffness in addition to other cues which can be used in differentiation to a special cell type and tissue engineering applications.
Keywords: Poly Acryl Amid, Cell Adhesion, Elasticity, Endothelial Cell, Atomic Force Microscopy

Ps-100: Muscle Cells Treated with Hypoxia and Laser *In Vitro* Can Recruit Mesenchymal Stem Cells through Overexpression of SDF1

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Objective: The applications of mesenchymal stem cells (MSCs) are still far away for being enlisted in the definitive and routine clinical settings. One of the most challenging problems is their inefficient homing to the desired damaged tissue. Recently, cell-based therapies have focused on novel strategies to solve this problem. Studies showed that stromal derived factor (SDF)-1 chemokine and its receptor, CXC chemokine receptor-4 (CXCR4), have pivotal role to direct homing of MSCs. Moreover, the effects of hypoxia and low level laser based treatments, which are well-known protective phenomenon for increased recruitment, retention and survival of transplanted stem cells at the site of injury have recently taken into consideration.
Materials and Methods: Here we evaluated the effects of hypoxic condition and low level laser (He-Ne) treatment on the human cardiomyocytes (hCMs), human skeletal myocytes (hSKMCs) and glioblastoma (as control positive) for expression of SDF-1. The second step was migration assay of MSCs toward these treated cells (hCMs, hSKMCs and glioblastoma); These parameters were analyzed by real-time PCR, western blotting and *in vitro* transwell migration assay, respectively.
Results: Results showed that hypoxia and low level laser treatment on hCMs have had the potential to induce overexpression of SDF-1 and subsequently increase the migration of MSCs toward pre-conditioning cells as compared with control groups.
Conclusion: Findings of this study elevate our knowledge about SDF-1/CXCR4 axis as an attractive target pathway to improve the beneficial impacts of homing and MSC-based therapies.
Keywords: Homing, Mesenchymal Stem Cells, SDF-1/CXCR4 Axis, Hypoxia Treatment, Laser Treatment

Ps-101: Effect of Crab Shell Extract on Nitric Oxide Production and Migration of Human Umbilical Vein Endothelial Cells

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Objective: Angiogenesis is the formation of new blood vessels from pre-existing vessels. There are many studies to decrease vessel growth in various diseases particularly cancers, including the use of natural compounds. Marine organisms have different therapeutic properties in various diseases. The aim of this study was to investigate the effect of crab shell ethanolic extract on nitric oxide production and migration of human umbilical vein endothelial cells.
Materials and Methods: Crab shell extract was first prepared, and human umbilical vein endothelial cells (HUVECs) were treated with different concentrations of crab shell (100, 200, 400, 800 and 1000 µg/ml) for 24, 48 and 72 hours. Nitric oxide production was assessed by Griess reagent and cell migration were evaluated in 400 and 1000 µg/ml concentrations. One-way ANOVA was used for data analysis. $P<0.05$ was considered significant.
Results: In all of the treatment groups, nitric oxide production by HUVECs was decreased in a dose- and time dependent manner ($p<0.05$). After 24 hours, a decrease in the migration of cells was also observed.
Conclusion: Crab shell extract decreases migration of human umbilical vein endothelial cells and maybe exerts this effect by decreasing of nitric oxide production.
Keywords: Migration, Crab Shell, Nitric Oxide, HUVECs

Ps-102: H2O2 as A Preconditioning Agent Increases Mesenchymal Stem Cells Sur-

vival: An *In Vitro* Study

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Objective: A growing number of experimental studies highlight the potential of stem cell transplantation as a novel therapeutic approach for diseases. But the massive loss of stem cells post-engraftment is an impediment that lessens the effectiveness of cell transplantation therapy. The present study tried to increase the survival of the stem cells by preconditioning them with H_2O_2.
Materials and Methods: Mesenchymal Stem cells were isolated from rat bone marrow. MSCs from passage four were cultured containing different concentrations of H_2O_2 (0, 5, 10, 20, 30, 40, .80, 90, and 100 μM) for 6, 12, 24, and 48 hours and then were recovered with fresh medium. Finally treated cells were exposed once to a lethal dose of H_2O_2 (300μM and 500μM).The viability of the cells were evaluated with trypan blue and proliferation assay with MTT assay.
Results: Preconditioning with 5μm H_2O_2 significantly increased the resistance of these cells against lethal conditions induced by H_2O_2 ($p<0.05$). MTT assay showed higher proliferation in groups treated with 5 and 10 μM H_2O_2 after 24 hours.
Conclusion: Preconditioning of MSCs with oxidative stresses enhances their survival and could therefore increase the efficacy of MSCs transplantation.
Keywords: Hydrogen Peroxide, Preconditioning, Cell Viability, MSCs

Ps-103: MicroRNAs in Breast Cancer Stem Cells and Their Potential for Breast Cancer Therapy

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Objective: Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death among women worldwide. According to the cancer stem cell hypothesis, breast tumors are hierarchically organized with cancer stem cells (CSCs). Targeting CSCs is of great interest as CSCs are considered to be more resistant to radiotherapy and chemotherapy. The small RNAs, which are called microRNAs (miRNAs), act as endogenous suppressors of gene expression, act as oncogenes or tumor suppressors, and are also thought to be responsible for the dissemination and growth of CSCs.
Materials and Methods: Aberrant expression of miRNAs was identified in cancer stem cells. Using stem-loop technology to test for differences in expression of miRNAs in breast cancer stem cells (BCSCs) and the MCF-7 breast cancer cell line; some miRNAs were over-expressed, with levels four times higher in BCSCs than in MCF-7 cells. These miRNAs included, miR-122a, miR-152, miR-212, miR-224, miR-296, miR-31, miR-373 and miR-489. Furthermore, miR-200a, miR-301, miR-188, miR-21, miR-181d, and miR-29b showed four times lower expression levels in BCSCs compared to MCF-7 cells.
Results: Taken together, it has been shown that above mentioned are involved in the proliferation or suppression of breast cancer stem cells. Regulating the expression of one specific miRNA could decrease the tumor-initiating, self-renewal ability of BCSCs or promote differentiation, which provides a promising new direction for clinical cancer therapy.
Conclusion: Finally, even though new technologies are emerging to improve the specificity, stability, and efficiency of miRNA delivery and therapy, the final outcomes remain uncertain.To fully reveal the mechanisms of miRNA function and to clinically apply miRNAs to tumor therapy, a substantial amount of further work is needed.
Keywords: miRNA, Breast Cancer Stem Cell

Ps-104: The Comparison of The Effect of Low Intensity Ultrasound and Low Intensity Pulsed Ultrasound on Proliferation, Colonization and Survival Rate on Neonate Mouse Spermatogonial Stem Cells

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Objective: Spermatogonial stem cells (SSCs) maintain spermatogenesis throughout the life of the male. Because of the small number of SSCs in adult, enriching and culturing them is a crucial step prior to differentiation or transplantation. Maintenance of SSCs and transplantation or induction of *in vitro* spermiogenesis may provide a therapeutic strategy to treat male infertility. This study compares the effect of low intensity ultrasound (LIUS) and low intensity pulsed ultrasound (LIUPS) on SSCs viability, proliferation and colonization.
Materials and Methods: Isolated SSCs from neonatal mice were cultured in DMEM culture medium with 10% Fetal Bovine Serum (FBS). Then, the identification of SSCs was confirmed by detection of PLZF protein in these cells and SSCs derived colonies. SSCs

were stimulated by LIUS and LIUPS for 5 days. To investigate the proliferation rate and colonization of treated cells, counting whole number of cells and colonies and their diameters were performed with an optical microscope at every seven days, respectively. The survival rate was assessed by MTT assay. Data analysis was done with ANOVA test.
Results: The LIUS and LIUPS treatment maintained the survival rate and increased the proliferation rate and colonization of SSCs during the first week of culture, but in the next weeks proliferation rate, colonization and survival rate were decreased in experimental groups.
Conclusion: These results suggest that LIUS and LIUPS treatment have short-term useful effect on SSCs proliferation and colonization during 21 days culture *in vitro*.
Keywords: Proliferation, Colonization, Mouse, Stem Cell, Ultrasound

Ps-105: Assessment of α6 and β1 Integrins Expression after Continuous and Pulsed Low Intensity Ultrasound on Mouse Spermatogonial Stem Cells

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Objective: Low intensity ultrasound (continuous and pulsed) is a form of energy. Spermatogonial stem cells (SSCs) maintain spermatogenesis throughout the life of the male. This study investigates the effects of low intensity ultrasound stimulation (LIUS) and Low Intensity Pulsed Ultrasound Stimulation (LIUPS) on the expression of germ cell-specific and pluripotency genes on SSCs during *in vitro* culture.
Materials and Methods: Isolated SSCs from neonatal mice were cultured in DMEM culture medium with 10% Fetal Bovine Serum (FBS). Then to confirm identification of SSCs, PLZF protein was detected in these cells and SSCs derived colonies. SSCs were stimulated by LIUS and LIUPS for 5 days then expression of α6 and β1 integrins (germ cell-specific gene) and Oct-4 genes (pluripotency gene) were assessed at day 21 and were analyzed by RT-qPCR. Data analysis was done with ANOVA test.
Results: The LIUS and LIUPS treatment of mouse SSCs increased the expression of α6 and β1 integrins genes in experimental groups compared to the control group ($p<0.05$), whereas there were not significant differences between groups regarding to expression of Oct-4 gene.
Conclusion: These results suggested that LIUS and LIUPS treatment had the good effect based on gene-specific marker expression.
Keywords: Integrin, Mouse, Stem Cell, Ultrasound

Ps-106: Definitive Endoderm Differentiation of Wharton's Jelly Mesenchymal Stem Cells Using Signaling Molecules in Three Dimensional Scaffold

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Objective: In this study, we focused on differentiating Wharton's Jelly mesenchymal stem cells into definitive endoderm lineage as the first step in differentiation into endoderm derivates, such as insulin-producing cells. Also, alginate capsules were utilized as a three dimensional scaffold. This system promotes cell to cell interactions that are essential for differentiation.
Materials and Methods: An alginate solution was generated by dissolving of sodium alginate powder in NaCl solution. To create a cell-alginate mixture, Wharton's Jelly Mesenchymal Stem Cells were added to this solution and capsules were generated by extruding the mixture in a $CaCl_2$ bath. Following encapsulation step, $CaCl_2$ was removed and replaced with cell culture medium. Cells were decapsulated during culture for viability analysis. In order to induce solubilisation, capsules were incubated with a solution containing sodium citrate, then Trypan blue exclusion method was applied to determine cells viability. Encapsulated cells have been induced to definitive endoderm formation by Activin A and Wnt3a. To investigate the expression of definitive endoderm related genes, the RNA was extracted and Real-time PCR was performed.
Results: The encapsulation procedure did not alter the morphology and viability of the enveloped cells. Post-differentiation analysis confirmed the expression of Sox17 and Foxa2, as definitive endoderm specific markers.
Conclusion: Our results showed that alginate has potential to be used as a three dimensional scaffold for culturing and differentiation of Wharton's Jelly mesenchymal stem cells to definitive endoderm. Also using signaling molecules such as Activin A and Wnt3a could enhance the definitive endoderm differentiation.
Keywords: Activin A, Alginate, Definitive Endoderm

Ps-107: Mathematical Modelling of Dynamic Culture in The Perfusion Bioreactor on A Three-Dimentional Model of Bone Formation

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Objective: The medium flow in the construct is essential for mammalian cells to grow and distribute uniformly in three-dimensional (3D) construct due to their specific nutrients' needs. Dynamic culture improves the distribution of nutrients and mass exchange which promotes the cell growth. Due to the complex and multiphase microstructure in the process, development of models for capturing the aforementioned knowledge is studied. The aim of this work was, the study of the effect of nutrient flow on cell growth in 3D scaffold.
Materials and Methods: A first the bone cells (designation: G292, species: human, national cell bank, Pasteur Institute, Iran) were cultured on a 3D sponge collagen scaffold (Matricel Company,Germany) for 24 hours in static culture and then the cultured scaffold was placed in a perfusion bioreactor with flow rate of 0.04 ml/minutes for 21 days. The scaffold was weighed in first and 21st days and the weight of the original scaffold was deducted from these weights. So, the ratio of cell density was obtained. The mathematical model proposed in this article included Brinkman, nutrient transport and cell growth equations and also permeability variation in the construct due to cell growth. Finally, the model was compared to the result of the experiment.
Results: As the result of the experiment showed, cell density was 53.5 times more numerous after 21 days; compared to the results of the model, it was 49 times. Consequently, the model has been almost validated.
Conclusion: This work has developed a mathematical model successfully that simulates the effect of nutrient flow on bone cell growth. The proposed model can be used for other dynamic culture to predict the amount and distribution of cell growth in 3D scaffold.
Keywords: Dynamic Culture, Mathematical Model, Bone Cells

Ps-108: Autophagy Modulation: A Key to Mesenchymal Stem Cell Survival Following Liver Cell Transplantation

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Objective: Therapeutic applications of bone marrow mesenchymal stem cells (BMCSs) as regenerative medicine "magic bullets" are limited due to their early death within the first few days following transplantation. This challenge resulted in development of several strategies to reinforce MSCs against lethal factors. In this study, we introduce a new strategy such as modulation of autophagy, a garbage disposal system in cell biology. We evaluated the effects of autophagy modulation in MSCs survival after transplantation to acute liver failure model in mice.
Materials and Methods: MSCs were isolated from discarded BM transplantation collection bags and filters. Autophagy was induced in MSCs by rapamycin. On the other hand, autophagy was inhibited through shRNA knockdown of ATG7 as autophagy crucial gene following cloning in DH5α E.coli. Induction or inhibition of autophagy was evaluated by GFP-LC3 puncta counting, western blotting and immunocytochemistry. In the *in vitro* phase, the response of MSCs following inhibition or induction of autophagy against lethal conditions were determined in terms of survival. Multilineage differentiation capacity of these MSCs were also evaluated. Then, in the *in vivo* phase, control and MSCs with inhibited or induced autophagy were transplanted to mice in which liver injured through CCl4 injection to determine whether modulations of autophagy would affect on repair. Finally, pathological and biochemical assays were performed to evaluate the regenerative potential of these MSCs.
Results: Autophagy inhibition increased survival of MSCs against unfavorable microenvironments such as hypoxia, serum depriviation and oxidative stress conditions. Moreover, inhibition of autophagy resulted in better recovery of injured liver. Interestingly, autophagy modulation had no adverse effect on MSCs multilineage differentiation potential.
Conclusion: Modulation of autophagy might act as a new strategy to improve the efficacy of MSC-based cell therapy at least in Liver injury.
Keywords: MCS, Autophagy, Acute Liver Failure, Mice

Ps-109: *In Vivo* Evaluation of Amniotic Membrane Extract (AME) Released from Chitosan Hydrogel in Partial Burn and Deep Wounds in Rat Model

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Objective: Human amniotic membrane contains different bioactive factors and has been used as a biological dressing to treat burn and skin ulcer. Also, its extract has similar characterization as is effective in the treatment of corneal diseases. Our previous results determined that amniotic membrane extract (AME) can promote epithelialization while suppressing stromal inflammation, angiogenesis and scarring. In this study we investigated the effect of AME released from hydrogel based on chitosan on partial and deep wounds in rat model.

Materials and Methods: We designed two partial burn and deep wound animal models (Wistar male rats 200-250g). After anesthesia, a 5×6 cm partial burn wound by 90°C water in 6 seconds was created in 22 rats. Deep wound model was created by removing 2×3 cm skin in 25 rats. Thereafter, hydrogel sheet was synthesized from chitosan-polyvinyl pyrrolidone (PVP) which included AME as a solvent of the hydrogel. The wounds were dressed by Hydrogel, Hydrogel contains 1mg/ml AME used up to 10th day then followed by 0.1mg/ml and Hydrogel contains 0.1 mg/ml AME as test groups. The control group was treated without any dressing. Biopsies were taken from wound and normal region for pathological evaluation in 10th and 15th days of experiment.

Results: Our results indicated that dressing of wound by Hydrogel contains 1mg/ml AME for 10 days and then it's changing with hydrogel contains 0.1mg/ml AME decreased the wound area ($p<0.01$) significantly, in compare with others in both wound models. Histological results confirmed that the amount of collagen fibers was increased and inflammatory cells were decreased in rats which were treated with hydrogel containing 1mg/ml AME and then followed by 0.1mg/ml AME in both models. In the other groups, acute inflammatory cells, fibrosis and scar were observed.

Conclusion: According to these results, we introduce an applicable dressing for partial burn and deep wounds which includes hydrogel containing 1mg/ml AME by changing it with 0.1mg/ml AME.

Keywords: Amniotic Membrane Extract, Wound Healing, Hydrogel, Burn, Rat

Ps-110: Neural Stimulation of Human Hair Follicle Dermal Papilla Stem Cells by Extremely Low Frequency Electromagnetic Field

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Objective: Chemical factors are among the most frequent signaling factors employed for stem cells differentiation into neural cells. Effect of physical factors on cell behavior recently has been proven. The aim of this study was to illustrate the effect of electromagnetic (EM) signaling on the differentiation of human hair follicle dermal papilla stem cells (DPCs) into neural cell by the evaluation of nestin (progenitor neural cells marker) and MAP2 (mature neuron marker) genes.

Materials and Methods: This study was designed to evaluate the neural markers' gene expression after 5 consecutive days treatment in 4 treatment groups defined as control, chemical, EM, and chemical-EM. To assess the role of extended treatment timing on neural differentiation markers m RNA levels, an EM group was prepared, treated for a time period of 14 days, and compared with a control group. In all groups, DPCs were cultured on 0.1% gelatin coated flasks. In the chemical tests, DPCs were cultured in DMEM medium containing EGF, bFGF, and DMSO while in the EM group, cells were cultured in DMEM medium and were exposed to EM field (1 mT, 50 Hz, 7hours/day). In the chemical-EM treatment group, DPCs were subjected to EM field together with chemical induction medium. After the treatment period, real-time PCR method was used to study the gene expression of nestin and MAP2.

Results: According to gene expression analysis, nestin gene expression levels in chemically stimulated group was shown to be 0.8265 fold, a 1.366 fold increase for EM (5d) treated group, a 3.9665 fold increase for the chemical-EM group and the EM (14d) treatment group showed a 1.7178 fold increase compared to the control group. Chemical induction generated 5.247 fold increase in MAP2 mRNA levels, whereas EM (5d) did not show any significant increase, no gene expression was detected for chemical-EM treated group and a 2.545 fold increase was observed in EM (14d).

Conclusion: Results from the chemically stimulated group are indicative of a high differentiation rate of DPC to mature neuron. The comparison between EM (5d) and EM (14d) results indicates that EM (5d) is at an early stage of differentiation. In EM (14d) group, the expression of MAP2 experienced a significant increase (2.545 fold) compared to a previous study (similar EMF parameters, but a 12 days continuous treatment). Lack of MAP2 gene expression in chemical-EM group seems to be attributed to the increase in intracellular reactive oxygen species (ROS) that leads to apoptosis. It has been shown that both epidermal growth factor and electromagnetic field increase ROS levels, however as progenitor neural cells have high level capacity of ROS, the significant increase in nestin expression in this group can be related to a cellular survival trend.

Keywords: Electromagnetic Field, Differentiation, Neural Cell, Nestin Gene, MAP2 Gene

Ps-111: Neural Differentiation of Human Bone Marrow Derived Stem Cells Using Real Time RT-PCR Technique

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Objective: Human adult stem cells which are capable of self-renewal and differentiation into other cell types can be isolated from various tissues. There are no ethical and rejection problems as in the case of embryonic stem cells, so they are a promising source for cell therapy. The human body contains a great amount of mesenchymal stem cells (MSCs). For the first time MSCs separated from bone marrow (BM) and between MSCs they are the most important one which are used in researches. The aim of this study was to explore the potential of human BM-derived stem cells (hBMSCs) in differentiating toward neural lineage.
Materials and Methods: To isolate hBMSCs, BM aspiration was cultured in DMEM: F12/10% FBS. The expression profiles of several MSC markers were examined by flow cytometry. After neurosphere formation, final differentiation was induced by B27, NEAA, L-glu and N2. Differentiated hBMSCs were evaluated for Nestin and MAP2 expression using Real time RT-PCR technique.
Results: Our results showed that BM-derived MSCs could be successfully differentiate into neuron-like cells as defined by cell morphology and up-regulation of neural marker MAP-2 expression in compare with undifferentiated cells.
Conclusion: Regarding to our results it seems that we can use BM tissue in order to investigate agents which consider having positive expected effects on neural cells' activities.
Keywords: Neural induction, Human BM-Derived Stem Cells (hBMSCs), Nestin, MAP-2

Ps-112: Using Western Blotting Technique to Study The Amount of MAP-2 and Nestin Proteins in Neural Differentiated Human Adipose Derived Stem Cells

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Objective: Human adult stem cells which are capable of self-renewal and differentiation into other cell types, can be isolated from various tissues. There are no ethical and rejection problems as in the case of embryonic stem cells, so they are a promising source for cell therapy. The human body contains a great amount of adipose tissue which contains high number of mesenchymal stem cells (MSCs) that can be easily obtained in large quantities by a less invasive method in comparison to bone marrow-derived MSCs. The aim of this study was to isolate, characterize and explore the potential of human adipose derived stem cells (hADSCs) in differentiating toward neural lineage.
Materials and Methods: To isolate ADSCs, adipose tissue was digested with collagenaseI and cultured in DMEM: F12/10% FBS. The expression profiles of several MSC markers were examined by flow cytometry. After neurosphere formation, final differentiation was induced by RA and BDNF. Differentiated ADSCs were evaluated for Nestin and MAP-2 proteins expression by using western blotting technique.
Results: MSC markers identified by flow cytometry in isolated ADSCs. Our results show that these cells could be differentiate into neuron-like cells as defined by cell morphology and expression of neural proteins (MAP-2 and Nestin) in compare with undifferentiated cells.
Conclusion: The present study demonstrated that we were succeeded in induction of ADSCs toward neural lineage and the expression levels of MAP-2 and Nestin proteins were increased.
Keywords: Neural Induction, Adipose-Derived Stem Cells (ADSCs), Map-2, Nestin

Ps-113: A Comparison between the Proliferation Rate of Stem Cells Derived from Human Bone Marrow and Adipose Tissue

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Objective: Mesenchymal stem cells (MSCS) are multipotent cells that can renew themself, proliferate and differentiated into a variety of cell types. In the last few years, human adipose derived stem cells (hADSCs), which can be easily obtained in large quantities by a less invasive method in comparison to bone marrow-derived MSCs (hBMSCs), have been suggested as an alternative source of pluripotent stromal cells. In this study, we attempted to compare the survival and proliferation potency of these cells with BMSCs.
Materials and Methods: After isolation of hADSCs and hBMSCs, their "stemness" was confirmed by flow cytometry. Viability and proliferation potency of these cells were evaluated using MTT assay.
Results: The present study demonstrated the viability and proliferation potency are nearly the same in both kind of stem cells we used.
Conclusion: According to our findings ADSCs could be a good alternative source for BMSCs and also a promising source of cell therapy.
Keywords: Adipose Derived Stem Cells (ADSCs), Bone Marrow Derived Stem Cells (BMSCs), Proliferation

Rate

Ps-114: Assessment of Human Embryonic Stem Cell Derived Neural Progenitor Cells Behavior on Nanofibrous Conductive Substrates

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Objective: Recent research has provided evidence that neural progenitor cells can sense their physical environment as they do in the case of chemical cues. The cell response to these physical properties such as stiffness, topography, electrical conductivity, etc would be considered as accelerated or reduced cell proliferation, differentiation toward a specific cell lineage and better *in vitro* functionality. In present study we will assess NPCs response to surface nanotopoghraphy and conductivity.
Materials and Methods: To achieve the above objective we used graphene and nano polycaprolactone (PCL) coated cover slips produced in Royan institute. Our experimental groups are graphene, polycaprolactone nanofibers and both graphene and PCL nanofibers compared with none coated simple cover slips.
Results: Primary results revealed undisputed cell alignment with nanofibers. Compared with $O4^+$ and $GFAP^+$ cells, higher population of $TUJ1^+$ cells was observed in immunostaining. Despite identical seeding cell population for all groups, in nanofibers and nanofiber$^+$ graphene coated plates higher number of cell were observed due to more proliferation or better cell survival. In the ongoing tests we are to prove which type of neuron is produced and check the impact of surface conductivity on functionality of produced neuron.
Conclusion: Cell alignment with nanofibers could help guided axonal growth in regenerative cell transplantation strategies. Considering higher population of TUJ1+ cells,it can be concluded that NSCs had more tendency to differentiate to neurons rather than Oligodendrocytes and astrocytes.
Keywords: Neural Stem Cell, Graphene, Nanofiber, Polycaprolactone

Ps-115: Increasing the Efficiency of Bone Chips in Non-Union Fracture Treatment Using Mesenchymal Stem Cells

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Objective: Cell therapy has been used in treatment of so many different diseases, recently. Osteodisease is one of them which has been studied a lot and a bunch of researches has been allocated to it. Non-union fracture is a subcategory of osteodisease and it usually appears in more than 20 percent of bone-feractures. The non-union fracture financial load is the result of treatment expenses and disablement which is caused by the disease. Ignoring the treatment of this kind of disease, would lead to some clinical problems including inflammation and tumor which itself, is the indicator of importance of finding an appropriate treatment. Nowadays, methods for non-union fracture treatment which has been used during operation are not so efficient.
Materials and Methods: In this study, bone chips are strengthened with the help of adipose tissue-derived mesenchymal stem cells (ASCs) to increase the reparation ability of bone fracture. First, non-union fracture is created in Rat, and then bone chip in combination with ASCs is applied to the place of bone fracture. The results have been considered by radiology, real-time PCR and histology.
Results: Morphological fracture healing was evaluated by radiographical and histological examination 8 weeks after surgery. To evaluate the fracture healing process, relative callus areas around fracture sites in scanned radiographs at different time point were analyzed. The radiological and histological results showed the improvement in reparation of cells in non-union fracture with the help of ASCs. To confirm the functional recovery of the fractured bone, biomechanical test was performed at week 8 in all groups. These findings indicated that incomplete union following bone fracture, repaired better in ASCs group than control group. Similarly, These results confirmed by real-time PCR.
Conclusion: Therefore, ASCs in combination with bone chips would be a promising approach to speed up and repair of non-union fracture.
Keywords: Mesenchymal Stem Cell, Bone Chips, Non-union Fracture

Ps-116: Generation and Characterization of Human Induced Pluripotent Stem Cells Derived from Diabetic Patient Using Polycistronic SOKM Non-Integrating Lentiviral System

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Objective: One of the promising approaches to understand and cure diabetes is to use pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Human iPS cells can potentially serve as a source for cell replacement therapy without the ethical obstacle of embryonic stem cells and would bypass immune rejection. Obtaining hiPS cells genetically identical to the patient presenting with diabetes has been a longstanding dream for the *in vitro* modeling of disease and ultimately cell therapy.
Materials and Methods: Induced pluripotent stem (iPS) cells were generated from Iranian diabetic patient by reprogramming their adult fibroblasts with four defined factors (Sox2, Oct4, Klf4, c-Myc) with the prospect of differentiating into insulin-producing cells to apply in cell replacement therapy. Following picking up the iPS-like colonies, confirmation tests including alkaline phosphatase staining, RT-PCR, real-time PCR, immunocytochemistry, embryoid body formation and *in vitro* differentiation were carried out.
Results: Fourteen days post infection of fibroblasts, hiPS colonies were picked. These cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens and gene expression which were indicated by high expression level of Alkaline Phosphatase, Nanog, SSEA4 and OCT4.
Conclusion: These iPS cells were shown to be similar to embryonic stem cells therefore having great potential for the use in regenerative medicine and for modeling genetic diseases.
Keywords: Induced Pluripotent Stem Cells, Nonintegrating Lentiviral System

Ps-117: Increase of GAG Secretion from MSCs in Response to The Functional Group Interactions of Amphiphilic Scaffolds

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Objective: One of the key challenges for any cartilage tissue engineering approach is providing adequate functional properties so that the engineered construct can mimic extracellular matrix (ECM) components of cartilage. This study focuses on determining the effect of the composition and cross linking of amphiphilic scaffolds on the glycosaminoglycan (GAG) secretion of mesenchymal stem cells (MSCs).
Materials and Methods: Scaffold preparation was based on mixing of aqueous and non-aqueous polymer solutions of polycaprolactone (PCL), polyvinyl alcohol (PVA), and gelatin (GEL) followed by homogenizing and freeze-drying. Then, the scaffolds were characterized by fourier transform infrared spectroscopy, and biological assays of MSCs culture, acridine orange/propidium iodide (AO/PI), and dimethyl methylene blue (DMMB) assay.
Results: The results from DMMB assay for GAG measurement revealed a significant increase in proteoglycan content for GEL/PCL/PVA scaffolds from 13.3905 ± 1.0429 µg/ml to 16.1767 ± 0.9917 µg/ml after 21 days. AO/PI results showed not only more than 90% of the cells remained alive inside the scaffolds but addition of GEL led to an increase in the number of the alive cells. More MSCs proliferated in the amphiphilic ternary scaffold containing GEL shows that the interactions between functional groups of GEL and PVA in cross linking process can provide a stable biomimetic microenvironment which stimulates MSCs for GAG secretion in cartilage tissue engineering.
Conclusion: The study revealed that the functional group interactions in cross linked GEL/PCL/PVA scaffold can increase the GAG secretion of MSCs.
Keywords: PCL, PVA, GEL, Cartilage, Cross Linking

Ps-118: The Effects of Pre- and Post- Ischemic Intra-Ventricle Injections of Mesenchymal Stem Cells on Brain Stroke in Rats

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Objective: Stroke is the third cause of death and first cause of disability around the world. Stroke could be resulted in systemic and focal inflammation in the ischemic area and it is responsible for an important part of neuronal loss. Expression of inflammatory cytokines, disrupting blood brain barrier, exposing central nervous system to peripheral immune system, up regulating of adhesion molecules are some results of inflammation after brain ischemia. Mesenchymal stem cells(MSCs) are one of the stem cells categories that could be isolated from different tissue such as adipose tissue, bone marrow, etc. MSCs are capable to reduce inflammation by secreting some anti-inflammatory cytokines such as

IL-10. MSCs can also regulate immune system. Therefore the aim of this project was to evaluate effects of intra-ventricular injection of MSCs on stroke in rats.
Materials and Methods: MSCs were obtained from fresh adipose tissue of the rats' abdomen after adding collagenase. Their multi-potency was confirmed by differentiating into adipocyte and osteocyte. The MSCs labeled with PKH26 and injected into lateral ventricle by stereotactic method before (pre-ishchemic group) and after (post-ischemic group) induction of stroke. The control group received PBS. Neurological assessments were done daily until 28 days. After fixation and sectioning, the brains were stained by HandE to determine the necrosis and ischemic area.
Results: The neurological assessment showed that clinical improvement in post-ischemic group was better than pre-ischemic and control groups after 28 days. Tracking of the Mesenchymal stem cells by PKH26 showed that the labelled MSCs migrate to cortex and striatum more than other areas in both treatment groups. HandE staining also showed that post-ischemic and control groups had the lowest and highest necrosis and ischemic areas, respectively.
Conclusion: Post-ischemic MSCs stem cell injection improved neurological signs and reduced ischemic and necrosis area more than pre-ischemic injection.
Keywords: Mesenchymal Stem Cells, Stroke, Intra-Ventricular Injection

Ps-119: Preconditioning of Bone Marrow–Derived Mesenchymal Stem Cells with H2O2 Improved Regenerative Potential of MSCs in Mice Liver Acute Injury

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Objective: Bone marrow–derived mesenchymal stem cells (BM-MSCs) are attractive cell sources for cell therapy. However, the efficacy of MSCs is limited because of low survival rate following translation. In this study, we preconditioned MSCs with sub-lethal doses of H_2O_2. We next examined the effects of preconditioning on the therapeutic potential of MSCs transplantated to acute liver injury in mice.
Materials and Methods: MSCs were isolated and cultured in low DMEM. Cells from passage four were plated at a density of 15000 cells/cm2 in 96-well plate and treated with 5, 10, 15, 20, 30, 40, 50, 60, 80, and 100µm concentrations of H_2O_2 (24 hours), and then were recovered in fresh medium for 7 hours followed by exposure to lethal doses of H_2O_2. Cell viability was measured with WST assay. Next, in the *in vivo* phase, we first induced acute liver injury then evaluated regenerative potential of conditioned MSCs. 48, 72, 96, and 120 hours later. Blood and liver samples were collected and analyzed using biochemical as well as histological methods.
Results: In the groups engrafted with pre-conditioned MSCs, survival rates was higher in comparison to who received normal MSCs. ALT and AST reached to normal level in the groups engrafted with pre-conditioned MSCs. Intrestingly, histological results revealed a significant improvement in liver regeneration potential in the groups transplanted with pre-conditioned MSCs.
Conclusion: Preconditioning of MSCs with oxidative stresses not only enhances their survival *in vitro*, but also increases the efficacy of MSC-based cell therapy in liver acute injury.
Keywords: MSC, Preconditioning, Oxidative Stress, Acute Liver Injury

Ps-120: Expression Evaluation of Two Members of DNA Repair System (OGG1 and MTH1) in Patients with Stenosis and In-stent Restenosis

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Objective: Coronary stenosis is known as one of the major life-threatening factors which is cured with variety of methods like Carotid angioplasty and stenting (CAS). With useful success, coronary restenosis occurred 4-6 months after percutaneous coronary intervention (PCI) still remains as a limitation for this procedure. The causative mechanism is not unraveled, despite all medical advances in order to find molecular basis of the process. Prior studies demonstrated the existence of DNA damages in coronary arteries and peripheral blood mononuclear cells (PBMCs). The aim of this study was to evaluate the expression of two critical members of DNA repair system as OGG1 and MTH1 for the first time.
Materials and Methods: Two groups of PBMCs consisted of 20 patients as stenotic group and 20 as patients with in-stent restenosis were purified. After RNA extraction, Real-time PCR was performed for OGG1 and MTH1 with beta actin gene as internal control.
Results: Kolmogorov-Smirnov test show normal distribution of data between two groups ($p<0.05$). Our Real-time PCR results confirmed the hypothesis that there is an expression difference between stenotic and restenotic patients
Conclusion: The study uphold that efficiency of repair system genes can influence the process of stenosis and also restenosis.
Keywords: DNA Damage, Gene Expression Differences, MTH1, Stenosis, OGG1

Ps-121: Association of XRCC1 Polymorphisms and Haplotype Frequencies with Risk of Developing Atherosclerosis

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Objective: Coronary artery disease (CAD) is the leading cause of death in all around the world. The main cause of CAD is the atherosclerosis of coronary arteries. Several evidences showed that DNA lesions may trigger CAD development. So, it has been proposed that single nucleotide polymorphisms (SNPs) that decrease DNA repair efficiency may play key roles in individual's susceptibility to atherosclerosis. One of the most important proteins which plays critical roles in different repair pathways is XRCC1. Two SNPs (Arg194Trp and -77T>C) were reported to affect function and expression of XRCC1, respectively. The aim of this study was to investigate the relationship between these two SNPs and the risk of developing CAD in Iranian population.
Materials and Methods: A case-control study was performed with 414 individuals including 207 patients diagnosed with CAD and 207 diagnosed as negative for CAD (control group). All participants were angiographically confirmed. SNPs were identified by the polymerase chain reaction–restriction fragment length polymorphism method.
Results: The XRCC1 Trp allele frequency for codon 194 was statistically more pronounced in the case subjects than in controls (OR=2.604, 95% CI=1.506–4.504, p=0.001). Another novel SNP -77T>C revealed protective role in our population through SP1 transcription effect (OR=0.618, 95% CI=0.399–0.960, P=0.032).
Conclusion: Taken together, our findings demonstrate a contributory role of XRCC1 SNPs in development of CAD. Furthermore, our results support the role of DNA damage and repair in cardiovascular disease.
Keywords: Atherosclerosis, Coronary Artery Disease, DNA Damage, Polymorphism, XRCC1

Ps-122: Seminal Vesicle Fluid Ameliorates Experimental Autoimmune Encephalomyelitis by Increasing FoxP3 Expression

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Objective: Thymus-arisen FoxP3 regulatory T-cells play pivotal role in preventing destructive immunity against auto immune response. These cells show reduced frequency and/or function in multiple sclerosis (MS). Insemination elicits expansion of natural FoxP3+regulatory T-cells pool at female reproductive tissue during mating due to seminal vesicle fluid. Notably, there was a differential recruitment of Foxp3+ regulatory T-cells into different organs with the lowest rate in brain than that of other organs. Such an effect on FoxP3 regulatory T-cells expansion prompted us to investigate effect of intra-CSF administration of seminal vesicle fluid on experimental autoimmune encephalomyelitis (EAE), the animal model for MS.
Materials and Methods: The disease was induced in female Lewis rats using guinea pig spinal cord and complete Freund's adjuvant. Seminal vesicles fluid was obtained from freshly slaughtered male rats. The animals treated with seminal vesicle fluid were considered as test group and two groups of rats were used as control in which one group was injected with saline and the second was left without injection. The animals were evaluated for weight loss and clinical signs from day 0 to day 14 after the disease induction. Expression of mRNA for IFN-γ, IL-4, IL-17, and FoxP3 in brain and spinal cord was determined using real-time PCR in which β-actin was used as reference gene.
Results: Administration of seminal vesicle fluid led to significant decrease in clinical sign, weight loss, and increase in Foxp3+ regulatory T-cells within brain and spinal cord, comparing with the control groups.
Conclusion: Our results suggest that seminal vesicle fluid contains all the required ligands for activation of Foxp3+ regulatory T-cells pool and has anti-inflammatory effect in organs other than reproductive organ.
Keywords: EAE, FoxP3-Treg, Seminal Vesicle Fluid

Ps-123: Growth Rate of Equine Marrow-Derived versus Fat-Derived Mesenchymal Stem Cells

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Objective: Two main sources for isolation of equine mesenchymal stem cells (MSCs) are bone marrow (BM) and fat tissue (FT) which are proper candidates for cell therapy in equine regenerative medicine. MSC populations isolated from different sources exhibit significant differences in their characteristics such as growth rate. Aim of this study was to investigate growth rate of BM-

MSCs versus FT-MSCs.
Materials and Methods: MSCs were isolated from fat tissue and bone marrow of 3 mares, cultured and passaged until passage3 (P3). Cells of each horse at P3 were seeded in 12 wells-plates with density of 30000 cells per well. After 24 hours, the cells of 3 wells were daily trypsinized, counted and averaged from day 1 to 8. Then, growth curve was plotted and population doubling (PDT) time was calculated.
Results: FT-MSCs and BM-MSCs became adaptive to the culture conditions during first 3 days and then started to enter the logarithmic phase (days 3-6). FT-MSCs after 7 days don't reach to plateau phase and their growth was continued with decreased rate. However, Logarithmic growth of BM-MSCs was finished at day 6 and cell population start to decrease at day 7. Mean of PDT was 43.82 and 78.15 hours for FT-MSCs and BM-MSCs, respectively.
Conclusion: Our finding indicates that growth rate of FT-MSCs is significantly faster than BM-MSCs *in vitro*. It means that the proliferation of FT-MSCs to reach appropriate number for transplantation purposes needs less time *in vitro*.
Keywords: Growth, Mesenchymal Stem Cells, Bone Marrow, Adipose, Equine

Ps-124: Transurethral Plus Transvaginaly Periurethral Injection of Autologous Adipose Stem Cells to Periurethral Region for Treatment of Female Stress Urinary Incontinence

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Objective: Stress urinary incontinence is a common medical problem among women. The urethral closure complex and/or the support mechanisms are responsible for incontinence in the majority of patients. Several surgical procedures with different degrees of invasiveness and outcomes have been reported to treat the problem.
Materials and Methods: Ten women with symptoms of stress urinary incontinence treated by injections of autologous adipose-derived stem cells into the periurethral region via transurethral and transvaginal went under urethroscopic observation. This report presents the short-term outcomes of the patients. Patients' outcome was evaluated by pad test results, ICIQ-SF scores and Qmax.
Results: The mean age of the participants was 45.8 ± 8.7 years. Urinary incontinence significantly decreased through the first two, 6 and 24 weeks after the injection therapy. The difference was significant in pad test results ($p<0.001$) and ICIQ-SF scores ($p<0.001$). Surprisingly, Qmax showed improvement after the study period (Means 32.6 vs. 35.7; $p=0.002$).
Conclusion: This study showed that injection of the autologous adipose-derived stem cells to periurethral region is a safe and effective treatment option for stress urinary incontinence.
Keywords: Stress Urinary Incontinence, Injection Therapy, Stem Cell Therapy

Ps-125: Effect of Chromium Chloride on Adipogenic Differentiation in Mouse Bone Marrow Mesenchymal Stem Cells

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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 adipocyte. Adipogenesis is a complex process that includes the proliferation of precursor cells, their commitment to the adipogenic lineage and terminal differentiation. Adipocytes deriving from MSCs *in vivo*, play a critical role in both normal physiology and the progression of various disease states. Chromium (cr3+) is an essential micro-nutrient and biochemical trace element in physiological functions. The aim of the present study was to evaluate the effects of different concentrations of chromium chloride on adipogenic differentiation in mouse bone marrow mesenchymal stem cells.
Materials and Methods: In the present study, mouse mesenchymal stem cells were isolated from an aspirate of bone marrow harvested from the tibia and femoral marrow compartment. Cells cultured in DMEM culture medium supplemented with 5٪ FBS for 72 hours. After the first passage cells were cultured in differential medium in group I (0.01 mg/ml insulin) and group II (0.01mg/ml insulin and 400 ng/ml dexamethasone) with different concentrations (0, 5, 10, 25 and 50μM) of chromium chloride (control and treatments I, II, III, IV and V, respectively) Cells were cultured for 21 days. The cell viability was assessed and adipose droplets formation was observed under light microscope. Sudan III and PAS staining were performed for detection of lipid and carbohydrate in the cells.
Results: Our results showed that the highest viability was in treatment I and the lowest viability was in treatment V in groups I and II. Microscopic observation established the formation of lipid droplets and carbohydrate storage in all treatments of both groups but intensity of stain was higher in group II, treatments IV, V.
Conclusion: Based on these observations, we conclude that the viability of cells in treatments reduced according to increasing the concentration of chromium chloride and with passing time.The result showed that

with the use of chromium chloride in medium at least can increase cell differentiation into adipocyte.
Keywords: BMSCs, Chromium Chloride, Adipocyte Differentiation

Ps-126: The Role of Sonic Hedgehog on Differentiation of Definitive Endoderm from Mouse Embryonic Stem Cells

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Objective: Sonic hedgehog (Shh) affects the development of embryo and also embryonic stem cells (ESCs). The aim of this study was to determine the effect of Shh inhibition during definitive endoderm differentiation of mouse embryonic stem cells.
Materials and Methods: The mouse embryonic stem cell line (MUKF3) was cultured for 5 days in non adherent petri dishes to form embryoid bodies (EBs). The media of control group (group I) contained DMEM/F12+%10 knockout serum replacement (KOSR). Experimental group (group II) received the hedgehog interacting protein (HIP) (10 µg/ml). Then EBs were cultured on gelatin coated dishes in attached position for further 8 days. Total RNA of each group was extracted and the expression of target genes was compared between groups by relative RT-PCR method and statistically analyzed. Also immunocytochemistry staining of Goosecoid and Sox-17 of treated EBs was done to identify the definitive endoderm cells.
Results: There was a significant decrease in expression of oct-4, nanog, brachury, shh, gata4 and goosecoid in the experimental group ($p<0.005$), while the expression of pdx-1 and tat in experimental group were significantly increased ($p<0.005$). Immunocytochemistry revealed that in the experimental group the amount of Goosecoid was lower in comparison to the control group but it seems that there was no difference for Sox-17.
Conclusion: The study presents Shh inhibition as a molecular pathway for induction of definitive endoderm from ESCs by affecting the fate of mesoendodermal cells.
Keywords: Definitive Endoderm, Hedgehog Interacting Protein, Mesendoderm, Mouse Embryonic Stem Cell

Ps-127: Differentiation of Human Adipose Derived Mesenchymal Stem Cells to Insulin-Producing Cells

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Objective: Diabetes mellitus type-1 or insulin-dependent diabetes is an autoimmune disease in which the beta cells of pancreas are attacked by the immune system and destroyed. To this date, no definitive way to cure the disease has been discovered. However, studies on the use of differentiated mesenchymal stem cells in the treatment of some diseases such as diabetes mellitus have emphasized. Evaluation of insulin-producing cell (IPCs) differentiation from human adipose mesenchymal stem cells (h-ASCs) in RNA and protein level is the main objective of this study.
Materials and Methods: Adipose tissue was obtained by liposuction. After enzymatic digestion and isolation of mononuclear cells, characterization of these cells was performed with differentiation assay to adipocytes and osteocytes and also cell surface specific antigens expression was detected by flowcytometry. Differentiation process was created in two stages. Low glucose-DMEM supplemented with 20% FBS, betamercaptoethanol (1 mmol/L) and nicotine amide (10 mmol/ L) was used in pre-conditioning stage for 48 hours. Then, the final stage includes high glucose-DMEM with nicotine amide (10 mmol/L) without FBS was performed up to 28th day. For expression assay of specific gene, RT-PCR and gel electrophoresis were done. Furthermore, dithizone (DTZ) staining and ELISA test for insulin secretion assay were performed.
Results: According to gel electrophoresis, expression of PAX4, Glut2, PDX-1, and insulin genes in mRNA level was observed. Bata-actin was performed as an internal control. ELISA data showed significant differences in insulin secretion at day 28 compared to day 14 as well as first day. DTZ-stained cellular clusters appeared after 28 days in the culture flask.
Conclusion: Cellular and molecular experiments for differentiation of h-Ad-MSCs to IPCs were evaluated in this study. Finally, based on the obtained results it can be suggested that this method is appropriate and functional for IPCs differentiation from h-ASCs.
Keywords: Adipose Derived Mesenchymal Stem Cells, Differentiation, Insulin-Producing Cells

Ps-128: Lectin Histochemisry Showed A Heterogeneous Population of Cells among Mesenchymal Stem Cells Isolated from Adipose Tissue

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Objective: Stem cells derived from adipose tissue as an appropriate source of mesenchymal stem cells (MSCs) have the potential to differentiate into multiple lineages. Glycoconjugates content of the MSCs can be considered as biomarkers determining the cells in self-renewal, pluripotency and differentiation stages. In this study, the lectin profile of MSCs isolated from adipose tissue was detected and according to that, a subpopulation was determined.
Materials and Methods: MSCs were isolated from adipose tissue by culturing the explant of the tissue. The FITC-conjugated lectins, WGA, UEA, PNA, BSA and Phy A were used to detect the terminal sugar residues. The cells were then counterstained with DAPI and were observed under the florescent microscope. The intensity of the reaction with lectins was scored with an arbitrary scoring system.
Results: MSCs were reacted with all lectins with different intensity of the reactions. The cells reacted with WGA, UEA, BSA "strongly " and with PHY, PNA" "very weak". The morphological analysis of the isolated MSCs revealed the existence of the two different cell types in the cultures. The cells with large nuclei were PNA-negative; however, they reacted with PHY, WGA and BSA with less intense reactions compared with cells contained smaller nuclei.
Conclusion: The MSCs derived from adipose tissue seem to be a heterogeneous populations and lectin profile of the cells showed they are different in the expression of the glycoconjugates. These two types of cells can be suggested to be separated based on their different lectin reactivity, especially by PNA.
Keywords: Adipose Tissue, Mesenchymal Stem Cell, Lectin, Glycoconjugate

Ps-129: Efficient Programming of Human Induced Pluripotent Stem Cells Derived from Fibroblast of Diabetic Patient to Definitive Endoderm-Like Cells

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Objective: Human-induced pluripotent stem cells (hiPSCs) are considered to be potentially able to differentiate into all human cell lineages and thus hold promise as an unlimited source for cell replacement therapies in clinical applications. Definitive endoderm (DE) formation is the first and crucial step in the development of visceral organs such as liver, lung, pancreas and so forth. Therefore efficient production of definitive endoderm is a prerequisite for efficient differentiation to mature endoderm derivatives.
Materials and Methods: Differentiation of hiPS cells to DE-like cells were carried out according the rotational suspension-based differentiation method introduced by Schulz on 2012. Expression Level of DE-specific markers including SOX17, FOXA2, and GSC as well as SOX7, Brachyury and SOX1 were analyzed by real time PCR and flowcytometery.
Results: The results indicated increase in the expression level of DE-specific markers including SOX17, FOXA2, and GSC and decrease in the expression level of primitive endoderm, SOX7 as well as mesoderm marker, Brachyury and ectoderm marker, SOX1.
Conclusion: Production of hiPS cell-derived definitive endoderm is a critical step in generating scientifically and therapeutically useful cells of the definitive endoderm lineage, such as hepatocytes and pancreatic endocrine cells.
Keywords: Human-Induced Pluripotent Stem Cells (hiPSCs), Definitive Endoderm (DE)

Ps-130: Anti-Inflammatory Effect of Adipose Derived Stem Cells

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Objective: Mesenchymal stromal cells (MSCs) are adherent and multipotent progenitor cells that have the capacity to self-renew and can differentiate into cells of several lineages. A wide range of evidences have demonstrated that MSCs have strong immunosuppressive effects on innate and adaptive immunity through various mechanisms. MSCs also have the ability to cause an arrest of cell division in the G0/G1 phase of the cell cycle. As a consequence of division arrest, MSC has been shown to affect, to a variable extent, T and NK cell cytokine secretion and cytotoxicity, as well as B cell maturation and Ab secretion.
Materials and Methods: Adipose derived stem cells (ASCs) were obtained from normal adipose tissue, in sterile condition, washed with PBS, cut in to small pieces, digested with collagenase type I and cultured in tissue culture flask containing MDEM 10% FBS. As cells reached passage 3, they were co cultured in two conditions, directly and by using transwell, with peripheral blood lymphocytes (PBLs) stained with Carboxyfluorescein succinimidyl ester (CFSE). The proliferation of CFSE-labeled PBLs was then analyzed by flow cytometry.
Results: Adipose derived stem cells caused 2-fold downregulation in CFSE-labeled-PBLs' proliferation.

This result was seen in both direct and indirect cultures.
Conclusion: Anti-inflammatory and immunomodulatory effects of adipose derived stem cells can introduce these cells as therapeutic interventions for a variety of immune related disorders.
Keywords: Anti-Inflammatory, Immunomodulatory, Mesenchymal Stromal Cells (MSCs)

Ps-131: Comparison of The Ability of Differentiation of Adipose Derived Stem Cells (ASC) to Osteocytes after Using Different Types of Composites Including Polyvinyl Alcohol-Gelatin-Hydroxy Apatite, -SiO_2, -ZrO_2 and Gelatin

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Objective: The management of large bone defects because of serious trauma, cancer, or congenital abnormalities is one of the major orthopedics problems. Tissue engineering can replace the damaged tissues using biologically compatible scaffolds along with suitable stem cells. The aim of this study was to compare the effect of different types of composites including Polyvinyl alcohol-Gelatin-hydroxy Apatite, -SiO_2, -ZrO_2 and pure gelatin on the differentiation potential of adipose derived stem cells (ASCs) to osteocytes.
Materials and Methods: The scaffolds were fabricated by electrospinning. Cell cytotoxicity and increase or decrease in cell number on different scaffolds was examined by MTT assay after 3 and 7 days post culture. Osteogenic differentiation was assessed by alkaline phosphatase (ALP) level as an early differentiation marker after 21 days at the end of mineralization period.
Results: MTT assay showed that ASCs were able to proliferate in all considered scaffolds. Polyvinyl alcohol-Gelatin-hydroxy Apatite and Polyvinyl alcohol-Gelatin-Sio_2 had the most and the least cytotoxic effects, respectively. Polyvinyl alcohol-Gelatin-hydroxy Apatite caused 23 and 33% decrease in cell viability at days 3 and 7, respectively. The osteogenesis assays indicated the superiority of Polyvinyl alcohol-Gelatin-Sio_2 in supporting ASCs for bone differentiation according to augmentation of cellular ALP levels.
Conclusion: The results of this investigation introduce Polyvinyl alcohol-Gelatin-Sio_2 scaffold as an appropriate candidate for bone generation in regenerative medicine.
Keywords: Tissue Engineering, Scaffold, Osteocytes Differentiation

Ps-132: Odontogenic Differentiation of Bone Marrow Mesenchymal Stem Cell by Low Intensity Ultrasound

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Objective: Dental disease affects human health and the quality of life of millions worldwide. In this study, the effects of low intensity continuous ultrasound (LICUS) were examined on differentiation of bone marrow mesenchymal stem cells to odontoblasts, the primary cell responsible for dentinogenesis and dentine repair.
Materials and Methods: Rat bone marrow mesenchymal stem cells (rBMSCs) were plated in a medium containing fetal bovine serum (FBS) and were cultured to passage 3. The cells divided to 3 groups: ultrasound, differentiation medium and ultrasound + differentiation medium. The cells were subjected to 1 MHz ultrasound at 355 mW/cm-2 . Cell viability, proliferation and odontogenic differentiation were assessed after the treatment with ultrasound.
Results: MTT result demonstrated that cells viability and proliferation in ultrasound group was higher than control group ($p<0.05$). Gene expression analyses demonstrated significantly increasing in the expression of odontogenic gens in ultrasound + differentiation medium group ($p<0.01$).
Conclusion: This study suggests that LICUS regulates proliferation and odontogenic differentiation of rBMSCs.
Keywords: Low Intensity Continuous Ultrasound, Bone Marrow Mesenchymal Stem Cells, Odontogenic Differentiation, Proliferation

Ps-133: A Novel Predicted Target for miR-126 Involved in Neurotrophin Signaling Pathway and Cancer Stem Cell Formation in Glioma

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Objective: Gliomas compose of ~30% of all brain and central nervous system tumors and 80% of all

malignant brain tumors. MicroRNAs (miRNAs) are small, single-stranded RNAs with an important role in the regulation of gene expression. miR-126 is found on chromosome 9 within intron 7 of the EGFL7 gene. IRS1 was significantly inhibited by upregulation of miR-126. IRS1 is capable to affect PI3K/Akt pathway through neurotrophin pathway. Neurotrophin pathway plays a significant role in neural cell differentiation. While KRAS gene implicated in the role of cellular differentiation affects PI3K/Akt pathway. Also, Ras and PI3K proteins involving the formation pathways of glioma cancer stem cells include Notch, Hedgehog (Hh), Wnt/beta-catenin and focal adhesion pathways. It is anticipated that upregulated miR-126 via downregulation of IRS1 caused glioma cancer stem cell. Here, we used molecular signaling pathway enrichment analysis approach to determine possible mechanism of association of miR-126 and IRS1 within neurotrophin pathway.

Materials and Methods: Validated and predicted targets (targetome) of miR-126 were collected of miRtarbase and miRwalk databases, respectively. Then, approximate expression of miR-126 targetome in glioma tissue was examined using Unigene database. Finally, in silico molecular pathway enrichment analysis was carried out by DAVID database to explore which of signaling pathway is related to miR-126 targetome and how miR-126 functions in the formation of glioma.

Results: Our data determined that some KEGG pathways including neurotrophin and focal adhesion signaling pathways are the most statistically associated with miR-126 targetome. Interestingly, four genes including KRAS, PIK3R2, IRS1 and CRK were influenced by miR-126. Among them, PIK3R2 and KRAS have the most effective role in focal adhesion and neurotrophin pathways.

Conclusion: According to our data, overexpression of miR-126 may lead to downregulation of IRS1 in the formation of glioma cancer stem cells.

Keywords: IRS1, miR-126, Neurotrophin Signaling Pathway, Cancer Stem Cell, Glioma

Ps-134: Early-Phase GVHD Gene Expression Profile in Target versus Non-Target Tissues: Kidney, A Possible Target?

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Objective: GVHD is a major complication after allo-SCT. In GVHD, some tissues like liver, intestine and skin are infiltrated by donor T cells while others like muscle are not. The mechanism underlying targeted tropism of donor T cells is not fully understood. In the present study, we aim to explore differences in gene expression profile among target versus non-target tissues in a mouse model of GVHD based on chemotherapy conditioning.

Materials and Methods: Balb/c mice were transplanted by Allogeneic B6 BM and splenocyte following Bu-Cy conditioning. Liver, kidney and muscle of animals were snap frozen at days -7, 0 and +7 following Allogeneic BMT (GVHD). Gene expression profile of these tissues was explored and compared.

Results: Expression levels of JAK–signal transducers and activators of transcription (STAT), CXCL1, ICAM1 and STAT3 were increased in the liver and remained unchanged (or decreased) in the muscle and kidney after conditioning. At the start of GVHD the expression levels of CXCL9, ITGb2, SAA3, MARCO, TLR and VCAM1 were significantly higher in the liver or kidney compared with the muscle of GVHD animals. Moreover, biological processes of inflammatory reactions, leukocyte migration, response to bacterium and chemotaxis followed the same pattern.

Conclusion: Our data show that both chemotherapy and allogenicity exclusively induce expression of inflammatory genes in target tissues. Moreover, gene expression profile and histopathological findings in the kidney are similar to those observed in the liver of GVHD mice.

Keywords: Gene Expression, GVHD, Mouse Model, Liver, Intestine

Ps-135: Hematopoietic Stem Cell Transplantation to Treat Multiple Sclerosis

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Objective: Traditional therapies for MS (IFN-β and glatiramer acetate) have achieved modest therapeutic benefits for patients with less aggressive MS but rarely reducing or reversing pre-existing disabilities. Now, more potent agents that interfere with immune function (natalizumab, fingolimod and etc.) have shown better activity at suppressing MS relapses but do not significantly reverse disability. Thus, autologous hematopoietic stem cell transplantation (HSCT) is proposed as a treatment for MS based on favorable results in experimental autoimmune encephalomyelitis.

Materials and Methods: This paper is a report of a review to evaluate the efficacy of HSCT in MS treatment and is prepared from 30 reputable articles. First,

HSCT protocol is presented, and then efficacy and results of this therapy are discussed.

Results: Since negligible HSCs are detectable in the peripheral blood, either a hematopoietic growth factor or chemotherapy is necessary to mobilize HSCs. HSCs are collected by apheresis. Blood is drawn from one lumen of the catheter, and mononuclear cells are separated by an external centrifuge and returned to the patient through the second catheter lumen. The PBSCs are further processed by immunoselection for a HSC phenotype and then cryopreserved. HSCs differentiate and replenish all types of blood cells.

Conclusion: The leading indication for autologous HSCT of autoimmune diseases is MS and over 500 patients with MS have undergone this procedure. Clinical studies showed a decrease of disease progression in almost 70% of cases, at least in the first three years of follow-up, and a dramatic reduction of relapses. The MRI studies demonstrated that autologous HSCT has the capacity of a profound and long-lasting suppression of gadolinium-enhancing lesions. New active lesions were rarely detected. Furthermore, the MRI studies showed that the volume of brain atrophy decreased significantly with time after 2–3 years following autologous HSCT. Nowadays, HSCT considers as a form of intense immunosuppressive therapy.

Keywords: Haematopoetic Stem Cell Transplantation, Multiple Sclerosis

Ps-136: Mechanical Vibration as A Physical Means to Influence Stem Cell Fate, Promoting Bone-Specific Gene Expression *In Vitro*

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Objective: Cells can sense and actively respond to mechanical stimuli. However, such active response promises a large potential in stem cells for future work in therapeutics tissue engineering. Recent studies introduce mechanical vibrations as a new mechanism in stimulating mesenchymal stem cells (MSCs). In the current study we tried to assess the effect of mechanical vibration on the bone-specific gene expression potential of MSCs *in vitro*.

Materials and Methods: To evaluate the effect of vibration loading on MSCs, the mechanical vibration device was designed and implemented at the Amirkabir University of Technology and has been patented in Iran. Bone marrow mesenchymal stem cells (BMSCs) were obtained from National Cell Bank of Iran. Cells were cultured in basal medium and were then subjected to vertical mechanical vibration at the frequency of 300 Hz, set for 45 minutes per day and for the extended period of 21 days. In order to quantify the osteogenic markers of the treated cells, gene expression of Runx2, and ColI was evaluated using a real-time PCR. Control cells were cultured similarly without vibration.

Results: RUNX2 is amongst the genes expressed during the early phase of osteogenic differentiation of MSCs. Results indicate that the vibration regime loading of 300 Hz increases the level of RUNX2 2.1 fold more than control group. COLI has a more effective role in the middle stage of differentiation and the vibration regime loading of 300 Hz increases the level of COLI 9.28 fold more than control group.

Conclusion: Our results are consistent with previous researches that introduce mechanical vibrations as a mechanism for mechanical stimulation of MSCs. This mechanical stimulus influences both the transcription and the production of extracellular matrix protein levels. Future clinical trials could benefit from the rapid differentiation of mesenchymal stem cells treated with non-invasive mechanical vibration.

Keywords: Mechanical Vibration, Mesenchymal Stem Cell, Osteogenic Differentiation, Tissue Engineering

Ps-137: Reducing The Risks in Producing Tissue Engineered Buccal Mucosa for Clinical Use

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Objective: Tissue engineered (TE) buccal mucosa has been used with good clinical outcomes in reconstructive surgery for the urethra. This involved the use of human acellular donor dermis, murine 3T3 fibroblasts as a feeder layer to expand oral keratinocytes and bovine foetal calf serum (FCS) to provide mitogens for these cells. Our aim was to avoid the use of donor human material, animal derived cells and sera in the production of TE oral mucosa, making it safer for clinical use. Our objectives accordingly were to replace human donor dermis with a biodegradable electrospun scaffold to be used as a synthetic dermal alternative and to replace mouse fibroblasts with screened human fibroblast as a feeder layer to culture oral keratinocytes and to avoid the use of FCS.

Materials and Methods: 10% PolyL-lactide (PLLA) in dichloromethane was electrospun to produce a scaffold. The human fetal lung fibroblast cell line MRC-5 was used to replace murine 3T3J2 fibroblasts or human oral fibroblasts were compared. In all cases oral keratinocytes were seeded into the scaffolds either in the presence or absence of FCS. In separate experiments media was supplemented with basic fibroblast growth factor (bFGF) or ascorbate-2-phosphate to increase collagen production. The results were assessed using AlamarBlue for cell viability and Sirius red to assess collagen production on days 7 and 14.

Results: Cells grew well on a 10% PLLA scaffold. We were able to expand oral keratinocytes in serum free conditions using either oral fibroblasts or MRC-5 fibroblasts as a feeder layer. The presence of bFGF or ascorbic acid-2-phosphate also increased collagen production.
Conclusion: We have achieved several steps to produce TE buccal mucosa which does not require donor human tissue, murine feeder cells or FCS and thus presents less risk of viral disease transmission for the patient.
Keywords: Tissue Engineering, Buccal Mucosa

Ps-138: The Study of Differentiation of Nurr1, Pitx3-Expressing Mouse Induced Pluripotent Stem Cells (iPSCs) into Dopaminergic- Like Cells

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Objective: Induced pluripotent stem cells (iPSCs) are considered to be potentially able to differentiate into all human cell lineages and thus hold promise as an unlimited source for cell replacement therapies in clinical applications. Parkinsons disease (PD) is a neurodegenerative disorder in which the dopaminergic neurons selectively lost. Due to the side effects of a dopamine precursor drug, cell replacement therapy especially using pluripotent stem cells and iPS cells for PD treatment has been considered recently.
Materials and Methods: In this study we established a mouse induced pluripotent stem cell (iPS) line co-expressed Nurr1 and Pitx3 transcription factors in order to efficiently generate DAergic neurons. Five stage protocol introduced by McKay's laboratory was carried out with some modifications for derivation of DAergic like cell. Expression Level of DAergic-specific markers including TH, MAP2 and NESTIN were analyzed by real time PCR and Immunocytochemistry and measuring of dopamine synthesis as well as its secretion by reverse-HPLC.
Results: The results indicated increase in the expression level of DAergic-specific markers including TH, MAP2 and NESTIN.
Conclusion: We report here that iPS cells can efficiently generate midbrain precursors and dopamine neurons. The result of this study may have impact on future stem cell therapy of PD.
Keywords: Induced Pluripotent Stem Cells (iPSCs), Dopaminergic (DAergic) Neurons

Ps-139: The Efficacy of Differentiation Potential of The Breast Milk-Derived Stem Cells into Hepatocyte, Cardiomyocyte, Neuron, Osteocyte and Adipocyte

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Objective: The human mammary gland as a dynamic organ that undergoes significant developmental changes contains mammary stem cells that are responsible for the changes during pregnancy, lactation and involution. Recently human breast milk can be considered as a novel non-invasive source of stem cells without any ethical controversy. The aim of this study was to evaluate the proliferation and differentiation potential of human breast milk stem cells (hBSCs) into hepatocyte, cardiomyocyte, osteocyte, adipocyte and neuron cell.
Materials and Methods: Human milk samples were collected in sterile tubes manually. The breast milk was diluted 1:2 with DMEM medium, centrifuged at 300g for 20 min. The cell pellet was washed twice with PBS containing 7% FBS. The cells were characterized for mesenchymal stem cell CD-markers by flow cytometry. To examine the pluripotency of human breast milk to three germ lineages, hBSCs were exposed to the hepatogenic, myogenic, osteogenic, adipogenic and neurogenic media. The immunofluorescence was performed to verify the differentiation of the hBSCs into various cell lineages.
Results: The cells expressed CD44 (88.3 ± 4.3%), CD45 (91.45 ± 506%), CD90 (41.6 ± 0.4%), CD271 (81.2 ± 5.8%), CD106 (9.5 ± 1.4%) and showed a negative reaction for CD34 (2.7 ± 2.1%), CD73 (3.8 ± 0.51%), CD105 (2.64 ± 0.55%) and CD144 (3.9% ± 0.95). The cells exposed to the hepatogenic media expressed CK19, CK18, alpha-fetoprotein and albumin. The myogenic-treated cells also reacted with troponin. The cells treated with osteogenic media were stained with alizarin S; however, the cells showed a weak potential for osteognic fate. The adipogenic-exposed cells were stained with oil red and the cells treated with neurogenic media expressed β-tubulin.
Conclusion: The hBSCs showed the potential to differentiate toward the derivative of three germ layers. Therefore, human breast milk can be considered as a non-invasive source of autologous source for cell therapy purposes.
Keywords: Human Breast Milk Stem Cell, Hepatocyte, Neuron, Osteoblast, Adipocyte

Ps-140: Evaluation of Liver Function

Tests (AST and ALT) in Patients with Hepatitis B and C in Tabriz

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Objective: Viral hepatitis is an infectious disease that causes liver damage and is one of the most important causes of mortality in the world. Annually more than one million deaths happen in the world due to viral hepatitis. The aim of the study was to evaluate liver function tests (AST and ALT) of patients with hepatitis B and C who referred to clinical laboratories in Tabriz city.
Materials and Methods: This cross study was conducted on 1000 samples from patients who had visited private clinical laboratories, infectious department, and dialysis . It was found that 693 individuals were hepatitis B carriers and 307 individuals were hepatitis C carriers.These samples were studied using standard methods (Elisa, Chemiluminescence, Photometer) to evaluate liver enzymes.
Results: Demographic data and results of enzyme levels were as follows: A. age range: 1 month up to 88 years old, B. the average age of the contaminated : 38+/- 6, C. the rate of infected men: 58%, D. the rate of infected women: 42%, E. liver tests of unnatural (AST, ALT): 27- 30%, F. the rate of contaminated people with both diseases: 2-3%, G. the rate of hepatitis B contagion in Tabriz: less than 0.3%, H. the rate of hepatitis C contagion in Tabriz: less than 0.15%.
Conclusion: The obtained results show that the percentage of contaminated males and females were different in comparison to global statistics which can be due to alcohol consumption, more addiction in males and less attendance of females because of some social problems. Therefore, careful observation and control specially in marriage course, pregnancy and the process of employment should be considered. In addition constant vaccination should be done. On the other hand, the best preventive measures can be taken by informing people about hepatitis disease .
Keywords: Hepatitis B and C, Liver Function Tests (AST and ALT)

Ps-141: Cancer Stem Cell Inactivation: Study of Stat3 Gene Expression in M2 Macrophages

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Objective: Immune cells in the tumor microenvironment not only fail to mount an effective anti-tumor immune response, but also interact intimately with the transformed cells to promote oncogenes actively. Signal transducer and activation of transcription Stat 3 gene expression in M2 macrophages increases tumor cell proliferation, survival and invasion while suppressing anti-tumor immunity as well as cancer stem cell activation. The persistent activation of STAT3 also mediates tumor-promoting inflammation. Genetic studies demonstrate that ablation of the Stat3 gene in either tumor cells or tumor-associated macrophages and other immune cells, even under chronic inflammatory conditions, inhibits carcinogenesis as well as the growth of established tumors.
Materials and Methods: In this study, murine macrophage J774 A.1 cell line was used as a typical mouse macrophage for evaluation of Stat3 gene expression in mRNA level. Culture condition followed by DMEM supplemented with 10% fetal bovine serum. Total RNA was extracted and converted to cDNA. PCR was performed by Stat3 primers and then gene expression was evaluated by gel electrophoresis. Beta-actin was used as an internal control.
Results: Gel electrophoresis data was shown Stat3 expression in murine J774 A.1 macrophage cell line.
Conclusion: Previous studies have been shown the ablation of the Stat3 gene in m2 macrophage caused to cancer stem cell inactivation. As regards Stat3 was expressed in J774 A.1 cell line thus it seems that this cell line can be used for knockdown studies in order to cancer stem cell inactivation.
Keywords: Cancer Stem Cell, STAT3, M2 Macrophages

Ps-142: The Effect of MiR-186 on Insulin Producing Cells

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Objective: Development of renewable sources of islet-replacement tissue for treatment of type I diabetes mellitus is an interesting worldwide issue. Placental tissue derived mesenchymal stem cells (MSCs) are a great promising source for regenerative medicine due to their

plasticity, and easy availability. MicroRNAs (miRNAs) are a class of small non-coding RNAs that play a central role in control of many biological activities. The miR-186 is expressed in the pancreas and is involved in islet development.

Materials and Methods: Human placental decidua basalis (PDB-MSCs) cells were cultured from full term human placenta. The imunophenotype of isolated cells was checked for CD90, CD105, CD44, CD133 and CD34 markers. The MSCs (P3) were chemically transfected with hsa-miR-186. Total RNA was extracted on the fourth and sixth day after transfection. The expressions of insulin, NGN3, PAX6, KIR6.2, PDX1 and Glucagon genes were evaluated by real- time qPCR on the sixth day.

Results: Flow cytometry analysis confirmed that more than 90% of cells are CD90+, CD105+, CD44+ and negative for CD133 and CD34. Morphological changes were followed from the second day, and during the sixth day cell clusters were formed.The expression of pancreatic specific transcription factors were remarkably increased during the four days after transfection and significantly increased on the seventh day.

Conclusion: The MSCs could be programmed into functional insulin producing cells by transfection of miR-186.

Keywords: MicroRNAs, MiR-186, MSCs, IPCs

Ps-143: The Effect of MiR-375 on Induced Pluripotent Stem Cells toward Islet-Like Cell Clusters

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Objective: Diabetes mellitus is characterized by either the inability to produce insulin or insensitivity to insulin secreted by the body. Islet cell replacement is an effective approach for diabetes treatment; however, it is not sufficient for all the diabetic patients. MicroRNAs (miRNAs) are a class of small non-coding RNAs that play an important role in mediating a broad and expanding range of biological activities, such as pancreas development. The present study aimed to develop a protocol to efficiently differentiate human induced pluripotent stem cells (IPS) into islet-like cell clusters (ILCs) *in vitro* by using miR-375.

Materials and Methods: The human iPS colonies were transfected with hsa-miR-375 by using siPORT™ NeoFX™ Transfection Agent. Total RNA was extracted 24 and 48 hours after transfection. The gene expressions of insulin, NGN3, GLUT2, PAX4, KIR6.2, PDX1, and OCT4 were then evaluated through Real- time qPCR.

Results: Morphological changes were observed on the first day after the chemical transfection and cell clusters were formed on the third day. The expression of pancreatic specific transcription factors was increased on the first day and they had significantly increased on the second day. The ILCs were positive for insulin and NGN3 proteins in the immunocytochemistry.

Conclusion: Overexpression of miR-375 can be an alternative strategy for producing ILCs from the iPS cells in a short time.

Keywords: Diabetes Mellitus, MiR-375, Pluripotent Stem Cells

Ps-144: Embryonic Stem Cells-Derived Cardiomyocytes in Suspension Culture as A Good Model for Evaluation of Doxorubicin Cardiotoxicity

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Objective: Nowadays highly efficient production of cardiomyocytes from human pluripotent stem cells is one of the most important subjects in stem cell research. These cardiomyocytes can be used for identifying developmental pathways, disease modeling, drug screening, and are ultimately used in clinical phases. So far, several protocols have been utilized for human embryonic stem cells (hESCs) differentiation into cardiomyocytes *in vitro*.

Materials and Methods: In the present study, it was tried to differentiate spheres generated from hESCs into cardiomyocytes in a define medium using small molecules. The physiology of mentioned cells has been evaluated by MTS assay for viability determination, flow cytometery analysis of DCFDH for ROS assessment, and morphological study using histologic microsections, in response to different doses of doxorubicin (0.03, 0.3, 3, 30μM), an effective anti-cancer drug- with cardiotoxicity side, effect.

Results: Results of the study showed that at doses of 3 and 30 μM of doxorubicin, the viability and beating of spheres was significantly reduced, and reactive oxygen species production was increased. In addition, cell morphology was changed.

Conclusion: The overall results obtained in the study showed that human embryonic stem cell-derived cardiomyocytes could be a useful model for *in vitro* evaluation of drug-induced toxicity.

Keywords: Doxorubicin, Embryonic Stem Cell, Cardiomyocyte, Cardiotoxicity

Ps-145: Repair of Bone Osteoporosis in Ovariectomized Rats by Using Zinc

Treatment

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Objective: Osteoporosis affects many people in the world and is associated with fractures, pain and disabilities especially for menopausal women so estrogen deficiency may lead to osteoporosis. Zinc (Zn) serves as a cofactor for many metalloenzymes involved in DNA transcription and protein synthesis and its deficiency can lead to such problems as impaired synthesis/secretion of FSH and LH. There are some interests in studies concerning the role of Zn in maintenance of bones. Zn may have a beneficial effects in preventing bone loss. The aim of this study was to investigate the potential effects of Zn treatment in protecting bone loss induced by ovariectomy in adult female rats.
Mature female Wistar rats weighing approximately 200 g were selected and ovariectomized. In control group rats were given distilled water and in experimental group were gavaged with Zinc (50 mg/kg). It was administrated for two months. The femur bone of the animals was collected and tissue bone investigated under a light and electron microscope level.
Results: The ovariectomized rats showed a significant decrease in bone mass density of femur in comparison with the intact control group. Ovariectomized group not treated by Zn showed disarrangement structure of lamella and uncalcified bone matrix. Administration of Zn reversed bone loss. It prevented destruction of bone tissue and arrangements were observed in the treated group had the same structure with contact rats compared to the ovariectomized group ($p<0.05$).
Conclusion: Zn prevents structural alternation in bone after osteoporosis. These findings suggest that Zn could have a potential effect as a new treatment for prevention of bone loss in postmenopausal osteoporosis.
Keywords: Osteoporosis, Zinc, Ovariectomy, Postmenopausal

Ps-146: Ultrastructure of Human Cumulus Oophorus: A Transmission Electron Microscopic Study on Vitrified Somatic Cells

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Objective: Results show that cumulus cells of the adult have the genetic content to direct the development of newborn. However, techniques involving fusion of embryonic or fetal somatic cells with enucleated oocytes have become steadily more successful in generating cloned young. Cryobiology is the study of the effects of low temperatures on living organisms. In the future, it may be possible to cryopreserve human cells, whole human organs. The aims of this study are to quantify the ultrastructural changes that occur in the cumulus cells during vitrification.
Materials and Methods: The study was divided into group A [(fresh cumulus cells (non-vitrified)] of human mature oocytes were used as controls) and B (cumulus cells of human mature oocytes were vitrified). Based on no significant differences in age, duration of infertility, infertile causes and transmission electron microscopy was used to compare the fine structure of vitrified cumulus cells of human mature oocyte and cumulus cells of human mature oocytes were non-vitrified.
Results: The ultrastructural changes cumulus cell showed that the mitochondrial membrane is normall as well as the Crista of mitochondria and the number of lipid droplets and vacuole have been increased in vitrified cumulus cell, while in our control group which is not vitrified, the membrane of the mitochondria remain intact and no vacuole is observed.
Conclusion: Vitrification did not damaged the organelles in vitrified cumulus cells Therefore, it is recommended to vitrify of cumulus cells for saving them permanently.
Keywords: Cumulus Cell, Vitrification, Ultrastructure, Somatic Cell

Ps-147: Assessment of Midkine Concentration and Anti-Inflammatory Cytokines in Multiple Sclerosis Patients Treated by Interferon Beta

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Objective: Midkine (MK), also called neurite growth-promoting factor 2 (NEGF2) is a basic heparin-binding growth factor. Inflammatory responses involve the release various mediators. Among these the MK has been known as an effective inducer of oncogenesis, inflammation and restoration of tissues. MK with stimulating effects in inflammatory responses through increasing the leukocytes migration in multiple sclerosis (MS) patients. The aim of this study was to evaluate the concentration of MK which associated to anti-inflammatory

cytokines.
Materials and Methods: To assess the MK levels and its association with anti-inflammatory cytokines (IL-10 and TGF-β) in multiple sclerosis patients treated by Interferon beta, the serum concentration of these proteins was assessed by ELISA in 32 MS patients in comparison with 32 healthy subjects.
Results: Based on our results, the MK concentration in MS patients is lower than healthy controls. Also we observed significant decrease in IL-10, and TGF-β cytokine levels in MS patients.
Conclusion: Our results approve a role for MK in inflammatory reactions in MS. In contrast to other animal study which stated MK increase in EAE model of MS, present research indicated that level of MK Serum decreased. This decrement seems to be due to Interferon beta treatment. Further studies need to be done in different MS patients to determine exact concentration of MK in these Patients.
Keywords: Multiple Sclerosis, Interferon Beta, Midkine

Ps-148: Evaluation of Adipose Mesenchymal Stem Cell Derived Neural Cells Exposed to Sera from Vitamin D Treated and Untreated Patients with Alzheimer's Disease

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Objective: Alzheimer's disease (AD) has considered in part a reflection of the ordinariness of this progressive neurodegenerative disorder in recent years. Mature amyloid plaques in this disease are frequently associated with numerous dystrophic axons and dendritic processes that lie within or immediately around the fibrous amyloid deposit. Exist of large numbers of such senile or neuritic plaques in limbic and association cortices is probably the single most reliable neuropath logical marker of the diagnostic entity. Researches have revealed that vitamin D deficiency is associated with increased risk of neurodegeneration, while vitamin D consumption due to antioxidant properties can restrict neural damage. So we aimed to compare neural gene expression in adipose mesenchymal stem cell (ADSCs) derived neural cells which treated by sera from AD patients.
Materials and Methods: We used sera from AD patients who treated and untreated by vitamin D for 3 months. First, HADSCs were isolated and differentiated to neuron like cells and then treated for two weeks with Sera from healthy individual (group 1), from AD patients who had not been treated by vitamin D (group 2) and from AD patients who treated by vitamin D for 3 months (group 3). Neurogenic differentiation of hADSCs evaluated by Quantitative real time polymerase chain reaction analysis (qRT-PCR).
Results: Evaluation of the percentage of neural markers, Nestin, GFAP and MAP2 determined by RT-PCR. After treatment, induced cells were not significant difference for Nestin expression, in comparison with the control group, while the mean percentage of MAP2 and GFAP expression was significantly decreased in vitamin D untreated group relative to the control group and group which treated by vitamin D ($p<0.05$).
Conclusion: Overall, present results and its comparison with literature studies confirmed vitamin D not only possess neuromodulator properties also preserve neural cells from damage which induced during neurodegenerative disease like as AD and Multiple Sclerosis. Different *in vitro* studies have been tried to advance the mechanisms reduction of neurotoxicity, in addition to identifying new strategies that have higher therapeutic efficiency in neurodegeneration.
Keywords: Alzheimer, ADSCs, Vitamin D

Ps-149: Nitric Oxid Effect on Endothelial Cell in Multiple Sclerosis Patients

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Objective: Multiple sclerosis is a progressive neuro demyelinative disease in which neural sheath and endothelial cell (EC) can be affected. In brain, functional changes in ECs contribute to reductions in resting blood flow. Furthermore, vitamin D3 has beneficial effects on endothelial dysfunction. This study presents direct experimental evidence associating free radicals and endothelial apoptosis as a basis of MS pathogenesis and response to vitamin D3 therapy.
Materials and Methods: Human umbilical vein ECs (HUVECs) were treated with sera from MS patients and sera from healthy volunteers (each group, n = 10). Free radical level was determined by NO Assay kit. Nitrite ($NO2^-$) levels were determined in the culture supernatants.
Results: Vitamin D3 prevented the production of Nitrite by the serum of patients. The mean ± SD of $NO2^-$ concentration showed significantly greater levels of dissolved NO2/NO3 metabolite in the culture media of untreated HUVECs by vitamin D3 as compared with control, while the rate of nitric oxide (NO) significantly decreased when vitamin D3 was presented in culture both in the pretreatment and in the post treatment group.
Conclusion: It could be concluded that EC treated with sera from MS patients activates apoptosis in HU-

VECs; this effect was reversed by vitamin D3 treatment. This can be proposed as a therapeutic approach for MS. In this study, we discovered that NO2 concentration and were significantly higher in the HUVEC media treated by MS serum as compared with the control, and elevation levels of dissolved NO2/NO3 metabolite was significantly reduced by co incubation of vitamin D3 in the pretreatment and post treatment groups of serum.
Keywords: Endothelial Cells, Multiple Sclerosis, NO2, Free Radicals

Ps-150: Ring Chromosomes: Do Etiology and Effects Meet ERVs?

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Objective: In patients with ring chromosomes compatible with life, phenotypic presentations are so complicated that introducing a vindictive rational genetic reason might be impossible. Recent studies highlighted reprogramming approaches might be applicable for correcting ring chromosomes during iPSC (induced pluripotent stem cell) procedures; hence plasticity hypothesis could open new doors in therapeutic era. Analyzing of whole genomes shows that endogenous retroviral elements (ERVs), which make up about 10% of mammalian genomes, are residues of an ancient infection and has been stabled and inherited in a Mendelian manner through generations.
Materials and Methods: Searching and alignment of ERV sequences throughout human genome by novel bioinformatics data analysis methods could lead us to develop a new approach for determination of active elements which integrated in critical harbors in genome.
Results: This is a theoretical hypothesis.
Conclusion: Although few data are available regarding the expression of ERVs in human tissues, we speculate that high frequencies of ERVs copy numbers per genome might be made them as an ideal gene expression candidate in assessment of molecular pathology of chromosomal structural abnormalities especially in patients with ring chromosome disorders. Based on recent data, there are unknown mechanism can be applied by reprogrammed cell to correct some chromosomal aberrations and we suggest that reactivation of ERVs function during reprogramming may facilitate generation of iPS cells without original chromosomal abnormalities.
Keywords: Ring Chromosome, Endogenous Retrovirus, Chromosomal Therapy, iPS

Ps-151: Influence of Nanoparticles of CdSe:ZnS on Mice Reproductive Systems' Development

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Objective: Quantum Dots (QDs) are ideal for development as luminescent probes due to the advantages of broadband excitation, narrow bandwidthemission and photochemical stability. Nevertheless, in vivo cytotoxicity of these nanoparticles has not been highly considered. This study aimed to evaluate the cytotoxicity effect of CdSe:ZnS on male reproductive system development.
Materials and Methods: 10, 20 and 40 mg/kg doses of CdSe:ZnS quantum dots were injected to some one month old male mice. Structural and optical properties of quantum dots were studied by X-Ray Diffraction (XRD), Scanning Tunneling Microscopy (STM) and the number of cells in seminiferous tubes of various groups were analyzed using SPSS 16.
Results: One month after CdSe:ZnS injection, studies of testis tissue showed a high toxicity of CdSe:ZnS in 20, 40 mg/kg dose followed by a decrease in basement membrane, destruction in interstitial tissue, deformation of seminiferous tubes, and reduction in number of spermatogonia, spermatocytes, spermatides, and matured sperms. Although histological study of epididymis tissue showed no significant effect of quantum dots on morphology and structure of tubule and its covering epithelium, there was a considerable reduction in the lumen sperm mass.
Conclusion: This study showed a high toxicity of CdSe:ZnS quantum dots on development of testis tissue, even in lower doses and considering lack of literature review in this field, this study can be an introduction to researches of toxicity effect of quantum dots on development of male reproduction system.
Keywords: CdSe:ZnS, Reproductive System, Development

Ps-152: *In Vivo* Toxicity CdSe:ZnS Nanoparticles on Kidney and Liver in Balb-c Mice

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Objective: Quantum dots (QDs) have been gaining popularity due to their potential application in cellular imaging and diagnosis, but their cytotoxicity has not been fully investigated. Studies about this subject are the most important investigations for using these nanoparticles in medical applications. This study aimed to evaluate the cytotoxicity effect of CdSe: ZnS on kidney and liver in embryo.
Materials and Methods: 10, 20 and 40 mg/kg doses of CdSe:ZnS QDs were injected to some female mice in day 8 gestation. Structural and optical properties of QDs were studied by X-Ray Diffraction (XRD), Scanning Tunneling Microscopy (STM).
Results: Histological studies of liver, kidney tissues in adult offspring showed high toxicity of CdSe:ZnS especially in 40 mg/kg dose which followed by degeneration in the cytoplasm and nucleus material in liver and degeneration of kidney tissue especially glomerular hypertrophy in a dose-dependent way.
Conclusion: On the whole, this study showed high toxicity of CdSe:ZnS on liver, kidney tissue, even in lower doses. Lately, QDs have been considered for photodynamic therapy of cancer. Considering results of this study about high toxicity, destruction in nuclear matter and their specific binding to the target, it seems that studies in this category can be very promising. Considering the lack of literature review in this field, this study can be an introduction to researches about histopathological effect of QDs on various organs.
Keywords: *In Vivo*, CdSe:ZnS, Kidney

Ps-153: The Expression and Function of Toll-Like Receptor 2 in Proliferation and Osteogenic Differentiation of Human Bone Marrow-Derived MSCs

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Objective: Adult stem cells with multipotent differentiation potential are present in almost all tissues of adult organisms. The main function of these stem cells is to support normal repair and rejuvenation of diseased and aging tissue. Multipotent mesenchymal stem cells (MSCs) seem to be a good candidate for cell therapy but information about factors controlling their proliferation and differentiation is not sufficient. Recently, studies have shown that chemokines and cytokines can affect MSC function. Toll-Like Receptors (TLRs), one of the main receptors in the innate immune system, can cause secretion of different cytokines like Interleukin 6 (IL6). Among TLRs, TLR2 has more different exogenous and endogenous ligands. In this study we investigated the expression of TLR2 during proliferation and osteogenic differentiation of human bone marrow MSCs (BM-MSCs).
Materials and Methods: MSCs were isolated from bone marrow of patients who were volunteers for cell therapy in Royan cell therapy center. The MSC identity of the isolated cells was confirmed based on the expression of surface epitopes including CD90, CD73, CD44, CD105, CD34, CD45, CD11b and CD31 and the differentiation capacity into bone, cartilage and adipose cell lineages. To examine TLR2 and IL6 expression pattern, we propagated MSCs in passage 3 (P#3) for three additional passages during which the expression of the TLR2 and IL6 were analyzed by qRT-PCR. Furthermore, bone differentiation culture conditions were established for P#3 MSCs and the expression pattern of TLR2 and IL6 was investigated at several time points including days 0, 7, 14 and 21.
Results: The expression levels of TLR2 and IL6 were decreased as the number of cell passages increased. Since the proliferation capacity of MSCs decreased with passage, these results indicate that there would be a direct correlation between TLR2 expression and MSC proliferation capacity. This was in accordance with our findings on MSC osteogenic culture. According to our observation, at osteogenic culture, TLR2 and IL6 were upregulated by day 7 (proliferation phase of differentiation culture) followed by their downregulation towards the end of the differentiation period. This observation also emphasizes the reverse correlation between TLR2 expression and MSC bone differentiation.
Conclusion: Taken together, our data indicates that there would be a direct correlation between TLR2 and IL6 expression and human MSC proliferation capacity and a reverse correlation between TLR2 expression and the MSC bone differentiation. These findings would be of importance for the field of stem cell therapy and regenerative medicine.
Keywords: Toll-Like Receptors, Mesenchymal Stem Cells, Osteogenesis

Ps-154: Effect of Storage Temperature on Cord Blood Hematology Parameters Over Time

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Objective: Standardized procedures for cord blood (CB) storage before and during cryopreservation as well as thawing are still controversial. This work is

concerned with the effect of CB storage temperature and storage duration through the stages before cryopreservation on cell recovery, viability, aggregation of cells and hematopoietic reconstitution potential.
Materials and Methods: Total of 30 CB samples were stored at 4°C, 12°C, 20°C, and 37°C for different periods of time after collection and before cryopreservation. Subsequently, these samples were compared in terms of recovery, viability, aggregation, and hematopoietic reconstitution potential.
Results: Duration and temperature used for CB storage affect cell recovery, viability, and aggregation of cells. Results were remarkably stable at 12-20°C for 48-72 hours.
Conclusion: CB cells demonstrate different degrees of vulnerability to *in vitro* cell death depending on the duration and temperature applied at the time of storage and before cryopreservation. Our results suggest that the best temperature before cryopreservation is 12-20°C up to 48-72 hours.
Keywords: Cord Blood Bank, Temperature, Hematopoitic, Duration

Ps-155: Transdifferentiation of Bone Marrow Stromal Cells into Cholinergic-Like Cells by Nerve Growth Factor and β-Mercaptoethanol

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Objective: Bone marrow mesenchymal stem cells (BM-MSCs) are pluripotent cells that have the ability of division, self-renewal and creation of a variety of differentiated cells such as neurons that can be used as a source of cells to repair damaged tissues. The aim of this study was to investigate the effects of nerve growth factor (NGF) and bone marrow stromal cells (BMSCs) on differentiation of BM-MSCs into cholinergic neurons by evaluation of OCT4 and Neuro D expression.
Materials and Methods: Mesenchymal stromal cells were isolated from bone marrow of adult rat long bone and cultured in α -MEM medium containing 10% FBS. After 5 passages, the expression of OCT4 and Neuro D was evaluated using PCR. The cells were pre-induced by BME for 1 day followed by induction with NGF for 29 days. Data was analyzed using ANOVA and Tukey post test.
Results: Expression of pluripotency marker Oct4 was increased during the pre- induction phase and decreased during the induction period,whereas Neuro D expression was not seen in pre-induction phase, but its expression increased along time in induction period.
Conclusion: These findings showed reasonable harmony between the increased expression of NeuroD and decreased expression of Oct4, that approved selected way to the differentiation of BM-MSCs to cholinergic-like cells.
Keywords: Bone Marrow Stromal Cells, Differentiation, NGF

Ps-156: Mechanical Stretch Stimulates Sdf-1α Expression in Skin Tissue and Recruits Circulating Bone Marrow-Derived Stem Cells to Expanded Skin

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Objective: Skin and soft tissue expansion is a procedure that stimulates skin regeneration by applying continuous mechanical stretching of normal donor skin for reconstruction purposes. We have reported that topical transplantation of bone marrow-derived mesenchymal stem cells (MSCs) can accelerate mechanical stretch induced skin regeneration. However, it is unclear how circulating MSCs respond to mechanical stretch in skin tissue.
Materials and Methods: MSCs from luciferase-Tg Lewis rats were transplanted into a rat tissue expansion model and tracked in vivo by luminescence imaging. Expression levels of chemokines including MIP-1α, TARC, SLC, CTACK, and SDF-1α were elevated in mechanically stretched tissues, as were their related chemokine receptors in MSCs. Chemotactic assays were conducted *in vitro* and *in vivo* to assess the impact of chemokine expression on MSC migration.
Results: MSC migration was observed in mechanically stretched skin. Mechanical stretching induced temporal up-regulation of chemokine expression. Among all the tested chemokines, SDF-1α showed the most significant increase in stretched skin, suggesting a strong connection to migration of MSCs. The *in vitro* chemotactic assay showed that conditioned medium from mechanically stretched cells induced MSC migration, that could be blocked with the CXCR4 antagonist AMD3100, as effectively as medium containing 50 ng/ml rat recombinant SDF-1α. Results from in vivo study also showed that MSC migration to mechanically stretched skin was significantly blocked by AMD3100. Moreover, migrating MSCs expressed differentiation markers, suggesting a contribution of MSCs to skin regeneration through differentiation.
Conclusion: Mechanical stretching can up-regulate SDF-1α in skin and recruit circulating MSCs through the SDF-1α/CXCR4 pathway.
Keywords: Bone Marrow Stromal Cells, Cell Migration, Mechanical Stretch, Chemokine, Skin Regeneration

Ps-157: First Step of Regeneration Intervertebral Disc by Synthesis Biopoly-

87 CELL JOURNAL(Yakhteh), Vol 16, Suppl 1, Summer 2014

mer Scaffolds and Identification Isolated Nucleus Pulposus Cells of Intervertebral Disc in Iran

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Objective: Low back pain is a major economical and social problem nowadays. Inter-vertebral disc herniation and central degeneration of disc are two major reasons of low back pain that occur because of structural impairment of disc. Inter-vertebral disc contains three parts as follows: Annulus fibrosus, transitional region and nucleus polpusus which forms the central nucleus of the disc. Reduction of cell count and extracellular matrix, specially in nucleus polpusis 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. In this study, proliferation of NP cells of human intervertebral disc compromised in chitosan-gelatin scaffold with alginate scaffold was studied.
Materials and Methods: NP cells derived from nucleus polpusus by collagenase enzymatic hydrolysis. They were derived from patients who undergoing open surgery for discectomy in Isfahan Alzahra hospital. Chitosan was blended with gelatin and glutaraldehyde was used for cross linking the two polymers. Then, alginate scaffold was prepared. Cellular suspension with 1× 105 transferred to each scaffold and cultured for 21 days. Cell viability and proliferation investigated by trypan blue and MTT assay. SEM was used to assert the porosity and to survey structure of scaffold.Eliza assay was used to assert production of extracellular matrix by NP cells.
Results: MTT assay and demonstrated that cell viability and growth of third day had significant difference in contrast by first day in both scaffolds. Accordingly, there was a significant decreased in cellular viability from day 3 to 21. Results of cell count showed a significant increase of cell numbers for alginate scaffold but there was no similar result for chitosan-gelatin scaffold. Result of SEM showed porosity of chitosan-gelatin scaffold and attached NP cells into scaffold.Eliza assay showed significant increase in production of extracellular matrix from day 3 to 14.
Conclusion: Alginate scaffold prepared a better condition for proliferation,viability and production of extra cellular matrix of NP cells in comparison with chitosan-gelatin scaffold. Results of this study suggest that alginate scaffold could be useful in in vivo studies and treatment.
Keywords: Intervertebral Disc, Degeneration , Scaffold , Chitosan , Alginate

Ps-158: Evaluation of Proliferation and Osteogenic Differentiation of Mesenchymal Stem Cell on PLLA (poly-l-lactide acid) Scaffold Nonamembrane

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Objective: Tissue engineering is combination of cells, engineering materials, and chemical and physical factors that its aim is improve or replace biological functions of tissue function in target tissues. scaffolds Is an important component of tissue engineering. These scaffolds are synthetic extracellular matrix used for cell attachment, proliferation and cell differentiation. In This project, the Nano membranes PLLA is used as scaffolds for proliferation and differentiation of bone cells.
Materials and Methods: Nano-phase separation method was used to production of PLLA membrane with a suitable stability and morphology. So we first obtained a homogeneous polymer solution and then stretched on a flat bed. After that, mesenchymal stem cells have been removed from dental pulp and were cultured on PLLA nano-membranes to differentiate into bone. In the end of osteogenic period, for evaluation of morphology, proliferation and differentiation quantitative Alizarin red staining, MTT assay, and measuring alkaline phosphatase activity, SEM investigation was performed.
Results: The results obtained in this project indicate that the nano-PLLA membrane expressed significantly greater alkaline phosphatase (ALP) activity ($p<0.001$), indicating the early promotion of osteogenic differentiation. the level of differentiation was evaluated with the help of quantitative Alizarin red ($P< 0.001$) . MTT assay demonstrated that the MSCs grown on scaffold nanomembrane had significantly Higher($P<0.001$) cell viability than the tissue culture control .PLLA nano membrane due to nontoxicity and good biocompatibility significantly enhanced proliferation and Osteogenic differentiation, several times in mesenchymal stem cells.
Conclusion: Nano PLLA membranes increases proliferation and differentiation of mesenchymal stem cell
Keywords: Scaffold, Mesenchymal Stem Cell, Proliferation, Differentiation

Ps-159: Repairing Effects of Allograft Bone Marrow-Derived Mesenchymal Stem Cells on Liver Fibrosis in Rat

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88 CELL JOURNAL(Yakhteh), Vol 16, Suppl 1, Summer 2014

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Objective: Despite its numerous limitations, liver transplants are the only definite cure for end-stage liver disease. The present study evaluated the hypothesis that intraperitoneally allotransplantation of bone marrow-derived mesenchymal stem cells can improve liver fibrosis in an experimental model.

Materials and Methods: Eighteen adult Wistar rats were induced liver fibrosis using thioacetamide (30 intraperitoneal injections biweekly) and were divided into three groups. Two groups were intraperitoneally allotransplanted with bone marrow-derived mesenchymal stem cells (106 cells/kg of body weight) 20 days after the last thioacetamide injection. They were euthanized 4 and 6 weeks after transplantation. The last group was selected as control and was euthanized 20 days after the last thioacetamide injection. Liver samples were histopathologically evaluated using Hematoxylin & Eosin staining and Masson's trichrome staining. Serum concentrations of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and albumin were compared between groups. Serum parameters were evaluated using one-way ANOVA and LSD post hoc test. Histopathologic scores were analyzed using Mann-Whitney test (SPSS 11.5). $P<0.05$ was considered significant.

Results: Transplanting bone marrow-derived mesenchymal stem cells improved survival, liver fibrosis and necrosis in the rats with thioacetamide-induced liver fibrosis. Bone marrow-derived mesenchymal stem cells also significantly recovered serum concentrations of alanine aminotransferase after 4 and 6 weeks (98.33±5.20 U/L and 89.67±3.85 U/L, respectively) and albumin after 6 week (2.93±0.12 g/dL) in comparison with control (132.0±17.95 U/L and 3.30±0.08 g/dL, respectively).

Conclusion: Allotransplantation of bone marrow-derived mesenchymal stem cells could ameliorate thioacetamide-induced liver fibrosis. This provides a novel approach for the treatment of fibrotic liver disease.

Keywords: Bone Marrow, Mesenchymal Stem Cell, Liver, Fibrosis, Rat

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