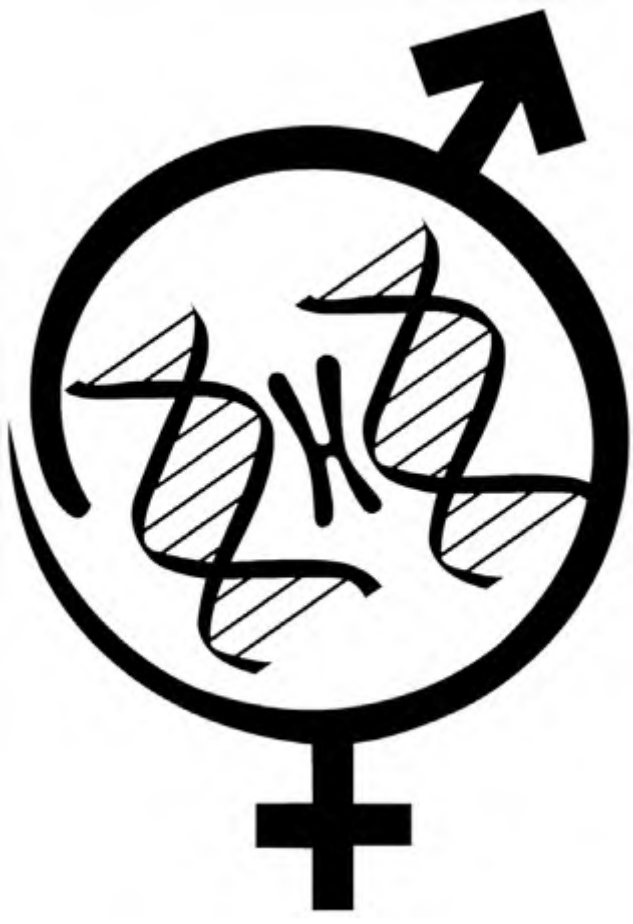
Abstracts of

Royan International Twin Congress

8th Congress on Stem Cell Biology and Technology

5-7 September 2012



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Cell Journal(Yakhteh)

Guide for Authors

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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 Manager of ***Cell Journal*** *(Yakhteh)*. May he rest in peace.

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Abstracts of the 8th Royan International Congress on Stem Cell Biology and Technology

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Congress Chairperson

Marzieh Ebrahimi

Dear Colleagues and Friends,

On behalf of the Organizing Committee, it is my great pleasure and honor to welcome you to the 8th International Congress on Stem Cell Biology and Technology, which is held in conjunction with the 13th Congress on Reproductive Biomedicine (Royan International Twin Congresses), September 5-7, 2012.

Royan Institute for Stem Cell Biology and Technology (RI-SCBT) as one of premier institutes conducting researches on Stem Cells is continually pursuing the aim to enhance research and development of stem cells and developmental biology for improvements in regenerative medicine. Therefore, RI-SCBT would like to give the opportunity for stem cell and biology scientists as well as physicians to debate and exchange their findings in a scientific and energetic atmosphere. To meet this goal, since 2005, we have annually held this scientific meeting.

The increasing number of participants and submitted articles during this event indicates the progression of stem cells among basic and clinic professions. Each year about 2000 participants, including enthusiastic young researchers and principal investigators from Iran as well as internationally, participate in this annual event. Moreover, internationally renowned expert scientists in stem cells research discuss their achievements during this program.

I encourage you to join us for the 8th International Congress on Stem Cell Biology and Technology held from September 5-7, 2012. I am convinced that this meeting will provide participants with unique experiences such as cultural encounters with Iranian tradition and history, as well as exchanges of the latest scientific knowledge in Stem Cells Research (Biologists and Physicians) from the scientific world.

Yours Sincerely,
Marzieh Ebrahimi, Ph.D.
Congress Chairperson
Stem Cell Biology and Technology Congress

Is-1: A Review and Update on The Current Status of Stem Cell Therapy in Royan Institute

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Currently, the vast majority of treatments for degenerative and or life threatening diseases are palliative. Cell or stem cell therapy holds great promise for improving human health by restoring cellular and organ function in these disorders. The filed of, stem cell therapy, had a relatively strong start in Royan institute, benefiting from institute scientific infrastructure.
After developing of Good Manufacture and Laboratory Practice to ensure production of sterile, potent and uncontaminated cells for clinical trials, Royan's first clinical trial, using autologous bone marrow derived stem cells, was done in myocardial infarcted patients. This study was followed by stem cell therapy trials in liver, skin, bone, joint, neurodegenerative, vascular and kidney disorders as well as progressively proceeding to the final phases of safety and efficacy. Now, there are more than 25 registered clinical trials by Royan institute in clinicaltrials.gov site. Clinical studies in Royan are focused mainly on the use of bone marrow and skin derived cells and there is no any clinical trial using embryonic or fetal stem cell.
This presentation reviews the current clinical outcomes about cell therapy in heart, bone and joint and skin disorders which are conducted in Royan institute.

Is-2: *Ex vivo* Stem Cell Approach to Retinal Degeneration

Ahmad I

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The identification and characterization of retinal progenitors with stem cell properties has opened new avenues that may be useful for treating functional impairments caused by the death of specific neural cell populations in the retina. Neuronal degeneration is the cause of debilitating visual impairment associated with prevalent ocular diseases, such as retinitis pigmentosa (RP), age-related macular degeneration (AMD), retinal detachment, and glaucoma. Retinal stem cells/progenitors may help to restore vision in patients who have these diseases by repopulating the damaged retina and/or by rescuing retinal neurons from further degeneration by ex-vivo cell therapy. However there are barriers to this approach, which primarily include sources of cells that can sustain a practical clinical use, free from immunogenic responses, tumor formation, and ethical burden. Significant progress has been made to overcome these barriers.

Is-3: Retinal Regeneration: Treating Retinal Degeneration from within

Ahmad I

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The discovery that the adult brain harbors stem cells that sustain neurogenesis throughout life has opened the possibility of treating degenerative changes from within by recruiting endogenous progenitors. This concept of therapeutic regeneration appeared remote for retinal degeneration because active neurogenesis has not been detected in the adult mammalian retina. However, neurogenic changes have been observed in injured adult retina, and the source of injury-induced neurogenesis is traced to Müller glia (MG). This and our recent observations that a subset of MG have evolutionarily conserved neural stem cell (NSC) properties posit these cells as a valid source of adult neurogenesis and therefore a target for therapeutic regeneration in intractable degenerative blinding diseases such as age-related macular degeneration (AMD). This approach could potentially address significant barriers, such as the lack of a renewable source of cells that are non-immunogenic and non-tumorigenic, which currently renders *ex vivo* cell therapy approach less pratical.

Is-4: Mechanisms on Arealization and Cell-Type Specification in The Developing Mammalian Neocortex

Alfano Ch[1, 2], Harb K[1, 2], Magrinelli E[1, 2], Elganfoud N[1, 2], Studer M[1, 2*]

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Corticogenesis involves the formation of six distinct layers and of functionally specialized areas characterized by specific sets of pyramidal neurons with distinctive morphologies, connectivity, and developmental programs of gene expression. We have previously shown that the transcriptional regulator COUP-TFI is required in balancing the neocortex into motor and sensory areas by regulating a genetic program leading to the correct differentiation of deep layer pyramidal neurons. We have now demonstrated that COUP-TFI controls areal identity and cell-type specification at the post-mitotic level. When COUP-TFI function is abol-

ished solely in early post-mitotic neurons by leaving its expression in progenitors unaffected, the primary and secondary motor areas, normally confined to the frontal cortex, expand to the occipital pole at the almost full expense of sensory areas. The transcriptional code specific for each cortical sub-population is altered in the absence of COUP-TFI function. In particular, expression of the LIM-homeodomain-related gene Lmo4 is highly upregulated in layers IV and V, while the bHLH transcription factor Bhlhb5 is downregulated in the same layers of the mutant somatosensory cortex. An increased number of pyramidal tract (PT)-type corticostriatal neurons is generated at the expense of the intratelencephalic (IT)-types in layer V of COUP-TFI mutant brains resulting in abnormal corticostriatal connectivity. Our study emphasizes the fundamental role of COUP-TFI in controlling areal and laminar identity by regulating expression of cell-type specific determinant genes at the post-mitotic level.

Is-5: De-Differentiation of Cardiomyocytes Is Instrumental for Cardiac Tissue Renewal after Myocardial Damage

Braun Th, Planck M

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Dedifferentiation is a common phenomenon among plants but has only been found rarely in vertebrates where it is mostly associated with regenerative responses such as formation of blastemae in amphibians to initiate replacement of lost body parts. Relatively little attention has been paid to dedifferentiation processes in mammals although a decline of differentiated functions and acquisition of immature, "embryonic" properties is seen in various disease processes. Dedifferentiation of parenchymal cells in mammals might serve multiple purposes including (i) facilitation of tissue regeneration by generation of progenitor-like cells and (ii) protection of cells from hypoxia by reduction of ATP consumption due to changes in energy metabolism and/or inactivation of energy-intensive "specialized" functions.
We recently found that an inflammatory cytokine of the interleukin 6 family, oncostatin M (OSM), initiates dedifferentiation of cardiomyocytes both *in vitro* and *in vivo*. Interestingly, activation of the OSM signaling pathway protects the heart from acute myocardial ischemia but has a negative impact when continuously activated thereby promoting dilative cardiomyopathy. OSM-mediated de-differentiation of cardiomyocytes results in a strong up-regulation of stem cell markers such as c-kit, and Runx-1. Furthermore, OSM-treated cardiomyocytes show an increased propensity for DNA-synthesis. The strong presence of the OSM receptor on cardiomyocytes and the unique features of the OSM signaling circuit suggest a major role of OSM for cardiac protection and repair. We propose that continuous activation or malfunctions of the cellular dedifferentiation machinery might contribute to different disease conditions.

Is-6: The Epistasis of Muscle Stem Cell Development

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Satellite cells are the stem cells of skeletal muscles. The mechanisms, which lead to a decline of the number and the fitness of muscle stem cells during life time are not understood. A stimulation of muscle stem cell renewal and differentiation holds an enormous potential to combat muscle-wasting disorders and to improve the clinical conditions of patients suffering from muscle dystrophies. We have approached several pathways that most likely control self-renewal, asymmetric cell division, and lineage selection of muscle stem cells. We also initiated studies to explore the heterogeneity of satellite cells, which are composed of distinct subpopulation of cells owing distinct capacities to regenerate skeletal muscle. One focus was on the transcription factors Pax7, Myf5 and MyoD, which seem responsible for the maintenance and/or renewal of muscle stem cells and for the acquisition of the fate of muscle stem cells. We have used a combination of genetic, cell biology and molecular biology techniques to access the epistatic regulation of the aforementioned regulators and devised new strategies for the manipulation of muscle stem cells. Another focus has been on the development of distinct myogenic lineages. Previous work had demonstrated that the development of myogenic cells is mainly determined by expression of two myogenic factors, Myf-5 and MyoD, which genetically compensate for each other during embryogenesis. More recently we demonstrated by conditional cell ablation in mice that Myf-5 determines a distinct myogenic cell population *in vivo* that also gives rise to MyoD positive cells. Ablation of this lineage uncovered the presence of a second autonomous myogenic lineage, which superseded Myf5-dependent myogenic cells. In contrast, ablation of Myogenin expressing cells erased virtually all differentiated muscle cells indicating that some aspects of the myogenic program were shared by most skeletal muscle cells. We concluded that Myf5 and MyoD define different cell lineages with distinct contributions to muscle precursor cells and differentiated myotubes. Individual myogenic cell lineages seem to substitute for each other within the developing embryo.

Is-7: Generation of Human Striatal Neurons from Stem Cells for Transplantation Studies in Huntington's Disease

Cattaneo E

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Medium-sized spiny neurons (MSNs) are the only neostriatum-projection neurons, and their degeneration underlies some of clinical features of Huntington's disease. Here we used human developmental biology and exposure to key neurodevelopmental molecules to inform human pluripotent stem (hPS) cells differentiation into MSNs. In a feeder-free adherent culture, ventral-telencephalic neuroectodermal specification is induced by BMP/TGF-β inhibition and subsequent morphogens treatment. The emerging FOXG1+/GSX2+ telencephalic progenitors are terminally differentiated, resulting in the systematic line-independent generation of CTIP2+/DARPP-32+MSNs. Similarly to mature MSNs, these neurons show *in vitro* inhibitory, spontaneous, and repeated spikes and dopamine neuromodulation, and synaptic integration ability *in vivo*. When transplanted into the striatum of quinolinic acid-lesioned rats, hPS-derived neurons survive, integrate, and are capable of differentiating into DARPP-32-expressing neurons, leading to a restoration of amphetamine-induced rotation behaviour. In summary, hPS cells can be efficiently driven to acquire a functional striatal fate using a stepwise method representing a platform for *in vitro* developmental neurobiology studies and drug screenings approaches.

Is-8: Using Stem Cells to Understand The Function of Disease-Genes: The Example of Huntingtin in Huntington's Disease

Cattaneo E

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Huntingtin (htt) is the ~800 million-year old protein product of the Huntington's disease (HD) gene. The gene contains a polymorphic tri-nucleotide CAG repeat that is translated into polyglutamine amino acid (polyQ) residues in the protein. When this polyQ stretch at the 18 aminoacid (aa) position of the protein expands to over 39 residues, HD occurs, a fatal, genetically dominant, neurodegenerative disease. The CAG repeats are well conserved in deuterostomes, which suggests that they are an ancestral feature retained during the evolution of the protein. Htt carries a number of specific activities in the adult brain; for instance, it promotes transcription of neuronal genes among which is the BDNF, a neurotrophin critical for the survival and activity of cortical and striatal neurons that degenerate in HD.
This presentation will highlight the power of combining evolutionary and developmental approaches to the study of the biology of disease-genes and will review the more recent discovery of a function for htt in neuroepithelial stem cells.

Is-9: Stem Cells in The Ovary and Their Potential Significance in Ovarian Ageing

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Is-10: Human Embryonic Stem Cells in Neurodevelopmental Toxicology

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Is-11: Differentiation of Human Embryonic Stem Cells into Neural Cells: Insights from Comparative Proteomics and Transcriptomics Analyses

Fathi A, Shahbazi E, Vakilian H, Baharvand H, Hosseini Salekdeh Gh

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Human embryonic stem cells (hESCs) are undifferentiated cells characterized by their functional capacity to both self-renewal and to differentiate into cell types representing the three embryonic germ lineages (e.g., ectoderm, mesoderm, and endoderm). Applying proteomics to explore the programs that control self-renewal, differentiation, and plasticity will provide valuable insight into ESCs biology. We used a microarray- and DIGE-based transcriptome and proteome approaches, respectively, to analyze hESCs differentiation to neural cells at three different stages: early neural differentiation, neural ectoderm, and differentiated neural cells. Owing to comparative analyses, several proteins and mechanisms emerged as key participants in stem cells proliferation and differentiation. Furthermore, using siRNA approach, we analyzed the function of two genes, MAGOHB and BCAS2, which were down-regulated at both transcript and protein levels during neural differentiation. The knockdown of these genesresulted in an increase in the expression of neural progenitor markers such as PAX6 and NESTIN. The knockdown of BCAS2 led to a decrease in NANOG expression level and an increase in the abundance of P53. The suppression of MAGOHB resulted in down-regulation of early endoderm and mesodermal gene markers, suggesting that it may play a role in enhancing neurogenesis through inhibition of endomesodermal formation.

Is-12: Overcoming Barriers to Successful Cell Therapy to Treat Liver

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Patients with life-threatening liver-based metabolic disorders require organ transplantation even though their metabolic diseases are typically the result of a single enzyme deficiency, and the liver otherwise functions normally. Hepatocyte transplantation holds great promise as an alternative to organ transplantation, and numerous studies in rodents indicate that transplants consisting of isolated liver cells can correct metabolic deficiencies of the liver. Consistent improvement in liver-based metabolic diseases has been reported in patients who have been treated by hepatocyte transplantation. Unfortunately, there have been no patients with complete correction of their metabolic enzyme deficiencies or evidence of long-term engraftment following hepatocyte transplantation. Based on extensive small and large animal studies, we have shown that conditioning part of the liver with low-dose focused radiation facilitates repopulation of the native liver by transplanted hepatocytes, can result in complete correction in models of hereditary metabolic deficiencies of the liver, and has led to the initiation of an FDA-approved clinical trial for the treatment of children and young adults with metabolic liver diseases. Unfortunately, the availability of human hepatocytes is limited as most cadaver donor livers are used for organ transplantation and the quality of human liver cells recovered from less-than ideal donors is often marginal. Patient-specific induced pluripotent stem (hiPS) cells have been created from a variety of somatic cell sources, and stem cell-derived hepatocyte-like cells can be generated with many of the features of primary human hepatocytes. Their potential for use as source cells for transplantation has been enhanced by studies showing that such cells can be successfully used to model alpha-1-antitrypsin deficiency *in vitro*, and can be transplanted in Gunn rats (a model of Crigler-Najjar syndrome type 1) to correct hyperbilirubinemia and generate conjugated bilirubin species in the bile.

Is-13: Use of Hepatocytes and Stem Cells in Understanding and Treating Liver Failure and Cirrhosis

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Hepatocyte transplantation holds great promise as an alternative to organ transplantation for the treatment of liver failure. In order to better understand the causes and potential barriers to treating liver failure from cirrhosis, we examined the extent to which organ function is affected in end-stage cirrhosis by damage to the native hepatocytes versus the abnormal environment in which they reside. We also examined the extent to which stem cells might play a role in functional recovery, and using microarray analysis, we identified HNF-4a expression as a critical factor in progressive hepatic dysfunction in cirrhosis. Rats treated with 14-16 weeks of CCL4 develop cirrhosis but have normal hepatic function, whereas animals treated with 26-28 weeks of CCL4 develop progressive liver dysfunction and die of liver failure approximately 6 weeks after receiving their last dose of CCL4. Primary hepatocytes derived from livers with advanced cirrhosis and compensated function maintain metabolic activity and the ability to secrete liver-specific proteins, whereas hepatocytes derived from cirrhotic livers with decompensated function fail to maintain metabolic or secretory activity. HNF4a expression is significantly decreased in liver tissue and in isolated hepatocytes from the animals with decompensated function. Cells from cirrhotic and control livers engraft equally well, but those from animals with cirrhosis and failing livers show little initial evidence of proliferative capacity or function. Both capacities, however, recover more than 2 months after transplantation, indicating that either mature hepatocytes or a subpopulation of adult stem cells are capable of full recovery in severe cirrhosis. More importantly, virus transduction to express HNF-4a in isolated hepatocytes from end-stage cirrhotic rats resulted in a dramatic improvement in expression of liver-specific genes and liver function. When the virus encoding HNF-4a was given IV to cirrhotic animals with decompensated liver function, hepatic function improved and survival was prolonged. Thus, the state of the host microenvironment is critical for tissue regeneration and restoration of function. As exogenous activation of HNF-4a by viral transduction causes reversal of hepatocyte dysfunction in advanced cirrhosis, disruption of this signaling pathway appears to be the mechanism responsible for hepatic failure in cirrhosis, and targeting this pathway may be partially effective in treating patients with end-stage liver failure from cirrhosis.

Is-14: Definition and Embryonic Origins of MSC-like Stem Cells in The Adult Mammalian Heart

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Objective: Identification of multi-potent stem cells in the adult mammalian heart has promoted a revision of the dogma that the heart is a post-mitotic organ with limited regenerative reserve, and stimulated clinical trials of stem cell therapies in patients with ischaemic cardiac disease, the most significant cause of death in developed countries. Our aim is to develop a quantitative framework for characterising mesenchymal stem cell (MSC)-like stem cells in the adult mouse heart, and to explore their role in homeostasis and potential for augmented regeneration.
Materials and Methods: A colony-forming assay (colony-forming units-fibroblast; CFU-F) was used to define cardiac MSC-like cells in normal and genetically modified mice. Formal lineage tracing of cardiac CFU-Fs was performed using bone marrow transplantation and Cre recombinase genetic technology.
Results: Cardiac CFU-Fs show long-term growth and are multi-potent for a variety of mesodermal and trans-germ layer cell lineages *in vitro* and *in vivo*, and have a gene expression and cell surface receptor profile resembling bone marrow (BM) MSCs. However, adult cardiac CFU-Fs do not arise from the bone marrow in health or disease. They have their lineage origins in an embryonic stem cell population termed the proepicardium, which is derived from the heart progenitor fields and forms the coronary circulation and other interstitial cells of the heart. BM, heart and aortic CFU-F arise from different post-gastrulation cell lineage compartments. Cardiac CFU-F can be stimulated by growth factor treatments that improve heart repair after myocardial infarction.
Conclusion: Our findings establish cardiac CFU-Fs as a stem cell population endogenous to the heart and likely dedicated to replacement of vascular, stromal and other cell types in homeostasis and repair. We propose that organ-specific CFU-Fs arise from the mesoderm and neural crest cells in organ-specific vascular beds, and understanding their organ-specific roles will open up new possibilities for regenerative therapies.
Keywords: Heart Failure, Heart Development, Stem Cells, Proepicardium, Mesenchymal Stem Cells

Is-15: Regulation of Quiescence and Multipotency in MSC-Like Stem Cells of The Adult Mammalian Heart

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Objective: Identification of multi-potent stem cells in the adult mammalian heart has opened up the possibility of stem cell and regenerative therapies for myocardial infarction (MI) and heart failure, which remain our greatest killers. In MI, endogenous stem cells may be lost or compromised by the hostile ischaemic and inflammatory environment, limiting the extent of natural repair processes. Understanding how cardiac stem cells are regulated by niche factors that could also be used to augment repair in the disease heart is now an important frontier. Using endogenous cardiac mesenchymal stem/stromal cell (MSC)-like cells as a model, we aim to explore the role of the platelett-derived growth factor (Pdgf) signalling system in regulation of stem cell states.
Materials and Methods: Endogenous cardiac MSC-like stem cells can be isolated from normal, genetically modified and/or diseased hearts and characterised using a variety of methods. Colony formation on tissue culture plastic in high serum is a semi-quantitative assay that defines the presence of these cells *in vivo*.
Results: Pdgf receptor alpha signalling appears essential for encouraging cells to emerge from their quiescent state after injury and may be an essential front-line response to ischaemic heart damage. It imposes its effects through regulation of both the cell cycle and the epigenetic state of chromatin across the genome with prolonged signalling favouring preservation of the multipotent stem cell state and epigenetic memory of this state.
Conclusion: Our studies suggest an avenue for enhancing an active MSC stem cell state in MI, and new cell and animal models for exploring the nature and stability of epigenetic states and transitions between different states in the stem cell hierarchy.
Keywords: Heart, Heart Failure, Myocardial Infarction, Stem Cells, Mesenchymal Stem Cells, Platelett-Derived Growth Factor

Is-16: Novel Approaches for Tracking Cancer Stem Cells

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We have shown that circulating invasive cancer stem cells (cCSC) in the blood are the root of metastasis and distinct from their non-invasive counterparts in the primary tumour. As most patients with advanced cancer eventually succumb from metastatic spread of the disease, it is of crucial importance to understand the distinct biology of cCSC and direct efforts for finding new therapies against this particular cell population. However, their prospective isolation from the blood is technically challenging. Rare cCSC have to be detected among all blood cells and also separated from non-tu-

mourigenic circulating cancer cells. Furthermore, their *in vivo* tumourigenicity as a defining feature of CSC must be demonstrated. To meet these criteria, we have developed a lab-on-a-chip microfluidic system based on immunomagnetic depletion of non-relevant blood cells followed by separation of cells according of their distinct physical characteristics. The efficiency of the system will has be validated using state-of-the-art *in vivo* mouse models for primary human cancers allowing the prospective isolation of circulating tumourigenic cells. While the specific detection of these circulating CSC can be used as a diagnostic tool for staging and monitoring treatment response, their prospective isolation from each individual patient provides a unique opportunity for tailoring therapies according to the features of individual cCSC.

Is-17: Identifying and Targeting the Achilles' Heel of Cancer Stem Cells

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Stem cells play a crucial role in the generation and maintenance of adult tissues, but emerging evidence suggests that they are also key elements in the development and progression of malignancies. Many solid cancers harbour a distinct subpopulation of cancer cells that bear stem cell features and are therefore termed cancer stem cells. Cancer stem cells are exclusively tumorigenic and essential drivers for tumour progression and metastasis. Tumours do not only contain one homogeneous population of cancer stem cells, rather than harbour diverse subpopulations including metastatic cancer stem cells that may have evolved during tumour progression. Clinically even more important, however, is the observation that cancer stem cells are highly resistant to chemo- and radiotherapy resulting in their relative enrichment during standard treatment and rapid relapse of the disease. By in-depth characterization of these cells in their biological context including the tumour microenvironment, we are aiming for a better understanding of their regulatory machinery. In this process, novel imaging, sorting and drug delivery modalities in conjunction with clinically most relevant cancer stem cells models are being utilized. These studies bear the potential to pave the way for developing novel platforms for targeted theragnostics and may eventually help improving the prognosis of patients suffering from these deadly diseases.

Is-18: Pluripotent Stem Cells for Toxicological Screening and Disease Models

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There is a high need to develop a novel toxicity test platform based on embryonic stem cells (ESCs), in particular human ESC (hESCs), to streamline the drug development RandD process and evaluation of drug toxicity in clinical studies, reduce related costs and thus to not only increase the safety of patients but also to reduce the number of animals due to earlier detection of adverse effects. The FP7 framework consortium ESNATS addresses current shortcomings in toxicity testing: It aims to develop a novel testing system taking advantage of the unique potential of hESCs, including their capacity to self-renew, constituting a potentially unlimited source of cells and their pluripotency, providing a source for cells of different phenotypes required for toxicity testing. Furthermore, hESC-derived somatic cells are physiologically relevant for toxicity endpoints, offering a perspective of tests with improved predictivity. To reach the project goals, a battery of toxicity tests has been developed using hESC lines subjected to standardised culture and differentiation protocols. Genomics approaches are used to determine predictive toxicogenomics signatures. The individual tests will be integrated into an "all-in-one" testing strategy. To ensure practical usage in the pharmaceutical industry, concepts for automated ESC culture will be developed and the test systems will be scaled up. The ESNATS project provides valuable information for risk identification in regulatory toxicology. Alternative testing strategies are highly needed in this field of work to limit the number of animal tests required to comply with the REACH. Moreover, human induced pluripotent stem cells (iPS) will provide additional tools to study the pathophysiology of monogenetic diseases enabling more distinctive and informative assay systems.

Is-19: Pluripotent Stem Cells from Basic Research to Possible Clinical Application

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It is our aim to provide a fundamental basis to the development of new medical treatments. This presentation will give an overview on our recent research work on human embryonic in comparison with induced pluripotent stem cells. Starting from basic investigations on the physiological properties of cardiomyocytes developed from pluripotent stem cells we have established *in vitro* and *in vivo* transplantation models enabling us to systematically investigate and optimize the physiological integration and regeneration of the diseased tissue. Our main focus is the cardiac infarction model. Induced pluripotent stem cell-derived cardiomyocytes (iPSCM) are regarded as the most promising cell type for cardiac cell replacement therapy. iPS cells are func-

tionally highly similar to embryonic stem (ES) cells, but in addition have the advantage of being ethically uncontroversial and obtainable from readily accessible autologous sources. A functional integration of iPSCMs is crucial for efficiency and safety, but has not been demonstrated, yet. Thus, we investigated the electrical integration of transplanted CMs into host tissue. Genetically modified murine iPSCM, expressing eGFP and a puromycin resistance under control of the alpha-MHC promoter, were purified by antibiotic selection. Purified iPSCM were injected into adult mouse hearts. At different times after transplantation recipients were sacrificed and viable ventricular tissue slices were prepared. Slices were focally stimulated by a u iPSCMs iPSCMs nipolar electrode placed in host tissue. Recordings of action potentials were performed by glass microelectrodes in transplanted iPSCM, which could be identified by their green fluorescence, and in host cardiomyocytes within the tissue slices.

Translation from the laboratory into the clinic is one of the key problems of stem cell research. Although proof of principle for the therapeutic use of iPS cells in cardiac diseases has been shown both at the laboratory scale and in animal models, the methods used today for generation, cultivation, differentiation and selection are not yet suitable for the clinic.

Is-20: Primate Totipotent and Pluripotent Cells

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Mammalian development commences with the totipotent zygote, which is capable of developing into all the specialized cells that make up a whole organism, as well as into the extraembryonic support structures necessary for fetal development. Early embryonic blastomeres, up to at least the 4-cell stage embryo, also retain totipotency. Pluripotent cells in the inner cell mass (ICM) of blastocysts are the descendants of totipotent cells and can differentiate into any cell type except some extraembryonic tissues of the trophectodermal origin.

Pluripotent cells can be isolated, adapted and propagated indefinitely *in vitro* in an undifferentiated state as embryonic stem cells (ESCs). ESCs retain their ability to differentiate into cells representing the three major germ layers: endoderm, mesoderm or ectoderm or any of the 200+ cell types present in the adult body. Since many human diseases result from defects in a single cell type, pluripotent human ESCs represent an unlimited source of any cell or tissue type for replacement therapy thus providing a possible cure for many devastating conditions.

Pluripotent cells resembling ESCs can also be derived experimentally by the reprogramming of somatic cells. Reprogrammed somatic cells may have an even more important role in cell replacement therapies since the patient's own somatic cells can be used to make stem cells thereby eliminating immune based rejection of transplanted cells.

The ability to contribute to chimeras upon reintroduction into host embryos is the key feature of murine totipotent and pluripotent cells. We recently demonstrated that rhesus monkey ESCs failed to incorporate into host embryos and develop into chimeras. However, ICMs transplanted into blastocysts formed separate viable fetuses while sharing the placental compartment of the host embryo. Classical monkey chimeras were produced by aggregation of totipotent cells of the 4-cell embryos.

Currently, there is little known about human and non-human primate embryo development and lineage specification and how closely the mouse development reflects primates. Our study presents a first glimpse at the similarities and differences between mouse and primate preimplantation embryo development and offers an important experimental model to investigate lineage commitment and interactions.

Is-21: Novel Reproductive Technologies for Preventing Mitochondrial DNA Diseases

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Mitochondria are critical for basic cellular function due to their principal role in the production of energy. Mitochondria contain their own genome (mtDNA) which, unlike the nuclear genome, is exclusively transmitted maternally from the egg. Mutations in mtDNA occur at a 10 to 100-fold rate higher than in nuclear DNA and cells usually contain a mixture of mutant and normal mtDNA, a phenomenon known as heteroplasmy. When the mutant load reaches a certain threshold, mitochondrial function is impaired leading to serious human disorders, including myopathies, neurodegenerative diseases, diabetes, cancer and infertility.

To establish preclinical models for new therapeutic approaches, we recently demonstrated that the mitochondrial genome can be efficiently replaced in mature metaphase II arrested rhesus monkey oocytes by chromosome transfer. Newly reconstructed oocytes consist of nuclear genetic material from one female and cytoplasmic components including mitochondria and mtDNA from another female. This approach yields developmentally competent oocytes suitable for fertilization and producing embryonic stem cells or healthy offspring. Potential clinical applications of this novel reproductive technology include mitochondrial gene replacement therapy to prevent transmission of mtDNA mutations and treatment of infertility caused by cytoplasmic defects in oocytes.

Is-22: Differentiation of Human Embryonic Stem Cells into Functional Renal Tubular Cells

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Objective: Proximal tubular epithelial cells (PTCs) perform a large variety of transport, metabolic and endocrinolgic functions in the kidney. Due to their role in drug transport and metabolism, this cell type is the major target for drug-induced toxic effects. Hence, PTCs are applied in *in vitro* nephrotoxicology and are also employed in bioartificial kidneys. Primary human cells are most interesting for both of these applications. However, the cell source is limited and interdonor variability is a point of concern. Human stem cell-derived PTC-like cells would be an attractive alternative, but protocols for the generation of stem cell-derived PTC-like cells have not been established. We have developed a protocol for the generation of PTC-like cells, and performed a thorough characterization of the cells.
Materials and Methods: In order to obtain PTC-like cells, human embryonic stem cells (hESCs) were cultivated for 20 days on Matrigel in renal epithelial growth medium with various supplements and growth factors. Subsequently, cells were characterized by *in vitro* and *in vivo* assays and in ex vivo organ culture.
Results: hESC-derived PTC-like cells formed epithelia *in vitro* and integrated into renal tubular epithelia in *ex vivo* organ cultures. The cells displayed the characteristic morphology and formed tubular structures *in vitro* and *in vivo*. Furthermore, PTC-like cells expressed various markers specific for PTCs and their precursors, and their gene expression patterns were remarkably similar to those of primary human PTCs. Markers specific for other types of renal cells were not expressed. PTC-like cells showed functional characteristics of PTCs, and they were functional in bioreactors mimicking the conditions in bioartificial kidneys.
Conclusion: PTC-like cells were successfully generated from hESCs. The usefulness of these stem cell-derived cells for applications in kidney tissue engineering and *in vitro* toxicology will be addressed.

Is-23: Cost-Effective Substrates for the Large-Scale Expansion of Human Stem Cells under Chemically Defined Conditions

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Objective: Clinical applications of human stem cells require large-scale expansion under chemically defined conditions. Appropriate *in vitro* culture systems should include synthetic substrates. Large-scale applications of expensive materials, such as peptide-based substrates, are cost-prohibitive.
Materials and Methods: Coatings consisting of polymerized 3,4-dihydroxy-L-phenylalanine (DOPA) were applied to flat synthetic membranes and synthetic microcarriers. Large-scale expansion of undifferentiated human embryonic stem cells (hESC), induced pluripotent stem cells (hiPSC) and mesenchymal stem cells (hMSC) on these substrates was addressed by using serum- and xeno-free media.
Results: The results revealed that the different types of human stem cells used could be propagated for up to at least 10 passages in the undifferentiated state on DOPA-coated susbtrates. Stem cell performance was comparable on commercial synthetic stem cell substrates and DOPA-coated substrates. DOPA-coated substrates are highly cost-effective in comparison to other synthetic substrates or Matrigel.
Conclusion: We have developed cost-effective, fully synthetic substrates for the large-scale expansion of various types of human stem cells under conditions that are free from components purified from humans or animals.

Is-24: Islamic Ideas about Moral Status of Human Embryo

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Research on human embryo is a controversy in around the world and there are different ideas about it according to different religions. Assisted reproductive techniques and production of numerous embryos, which can be cryopreserved for years, has raised a complex situation about using human embryos in research. Embryonic stem cells and hope of treatment of incurable disease made it even more complex. Iran is the only Islamic country that practices gamete and embryo donation programs and also has human embryonic stem cell lines. Basic points are respect, individuality and personhood of the human embryo. Here, I presented some Islamic idea about the moral status of human embryos, using the embryos in research and therapeutic abortion. The presentable Conclusion are as follows:
1. Pre implantation embryo is not considered as human or potential human and can be used in research with the permission of the owners of the embryo. Islam has the border of implantation for considering the embryo as a potential human. Before implantation, embryos are just respectable because they have human origin like human organs.

2. Individuality of the embryo is not a religious issue. Biologically, after 14 days the embryo cannot split and make identical twins, its three layers are appeared and begins to form the neural system. So, the 14 days of embryo's age is the border of individuality.
3. After implantation, although the embryo is not considered human, but is respectful. Any manipulation of embryo in the uterus or abortion in any gestational age is considered a sin and has its own penalty.
4. Personhood in many religions is related to the human soul, so, ensoulment time is considered the border of personhood. After 120 days (for Shiaa Muslims) and 50 days (for Sunni Muslims), it is believed that ensoulment of the fetus happens, so fetus is considered a complete human, and no abortion is allowed unless the mother's life is in danger. The personhood time is different between religions like after birth for Judaists, conceptions for Catholics and 14 days for some protestants.
5. According to shiaa decrees, before ensoulment, the therapeutic abortion is allowed if there is an absolute medical reason. Therapeutic abortion is defined as when the fetus has abnormalities or diseases or the pregnancy has a great burden for the mother. The basis of this decree is the burden of a disabled child on the parents and the society and also the suffer of child itself. If the life of the mother is in danger, pregnancy termination is allowed in any gestational age.

Is-25: Generation of Transgenic Reporter Lines in Human Pluripotent Stem Cells for Cardiac Subtype Specification

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Pluripotent human embryonic stem cells (hESC) have the potential to differentiate to any cell type of the human body. This characteristic has sparked researchers to study the use of hESC for regenerative medicine, drug screenings and embryonic development. We have recently optimized differentiation of hESC to cardiomyocytes, including growth factor directed differentiations as monolayers or as three-dimensional aggregates (embryoid bodies or EBs). Previously, we have demonstrated that hESC-derived cardiomyocytes (hESC-CM) faithfully recapitulate the early molecular events during embryonic development. Recently, we have generated a cardiac reporter line by introducing Green Fluorescent Protein (GFP), in the genomic locus of the early cardiac transcription factor NKX2-5, which enables us to visualize the derivation of NKX2-5+ cardiomyocytes during *in vitro* differentiation and purify these cells by Fluorescent Activated Cell Sorting (FACS). The combination of different transcription factor-coupled fluorescent reporters in this so-called "rainbow" hESC cell line, covering sequential stages of the cardiac lineage, will allow us to identify and characterize pathways for specific subtypes of the cardiac lineage at early and later stages during differentiation. Furthermore, a better understanding of these developmentally related processes will be further important for progress in fields of tissue engineering, disease modelling, drug toxicity and discovery, which most likely will lead to improved tailor-made therapies and better and safer medicines on the market.

Is-26: Human Stem Cells for Cardiac Disease Modeling and Drug Screening

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In drug discovery and development it is of upmost importance to distinguish target and off-target effects. The process from target discovery to delivery of safe and effective drugs takes many years and is very expensive. The percentage of drugs that actually make it to the market is extremely low. Before drugs reach the market they need to be tested on their safety. This is particularly relevant for the heart where off-target effects can lead to disturbance of the heart rhythm, which may lead to fatal arrhythmias. Many of these drugs act by extending the QT interval, the time between polarization and depolarization in the ventricle, thus, by definition, are potentially high risk since QT extension can itself lead to fatal arrhythmias. Therefore all drugs need to be tested on the prolongation of the QT interval of the action potential. One important goal in preclinical screens is therefore to identify compounds which extend QT. Current *in vitro* models are artificial whereas results from screenings in different species can not directly be extrapolated to the human situation. It is thought that the use of human cardiomyocytes for screening drugs may provide a more predictable model and could partially replace existing *in vitro* and *in vivo* models. Furthermore, we will discuss cardiac disease modelling and the use of cardiomyocytes derived from human stem cells on different classes of drugs and the predictability of these models.

Is-27: Human Induced Pluripotent Stem Cells Differentiation into Oligodendrocyte Progenitors and Transplantation in A Rat Model of Optic Chiasm Demyelination

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Objective: This study aims to differentiate human induced pluripotent stem cells (hiPSCs) into oligodendrocyte precursors and assess their recovery potential in a demyelinated optic chiasm model in rats.
Materials and Methods: We generated a cell population of oligodendrocyte progenitors from hiPSCs by using embryoid body formation in a defined medium supplemented with a combination of factors, positive selection and mechanical enrichment. Real-time polymerase chain reaction and immunofluorescence analyses showed that stage-specific markers, Olig2, Sox10, NG2, PDGFRα, O4, A2B5, GalC, and MBP were expressed following the differentiation procedure, and enrichment of the oligodendrocyte lineage.
Results: These results are comparable with the expression of stage-specific markers in human embryonic stem cell-derived oligodendrocyte lineage cells. Transplantation of hiPSC-derived oligodendrocyte progenitors into the lysolecithin-induced demyelinated optic chiasm of the rat model resulted in recovery from symptoms, and integration and differentiation into oligodendrocytes were detected by immunohistofluorescence staining against PLP and MBP, and measurements of the visual evoked potentials.
Conclusion: These results showed that oligodendrocyte progenitors generated efficiently from hiPSCs can be used in future biomedical studies once safety issues have been overcome.
Keywords: Human Induced Pluripotent Stem Cells, Oligodendrocyte Progenitors, Transplantation

Is-28: Regulation of Embryonic and Adult Neural Stem Cells during Mouse Corticogenesis

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Area-specific cytoarchitecture implies a tight spatiotemporal control on the lateral expansion of individual cortical areas coupled to radial growth, which will determine cortical thickness and cell-type specification. The mode of neural-progenitor cell divisions influences cortical expansion and growth. While progenitors initially divide symmetrically to increase the stem cell pool, asymmetrically dividing radial glial cells will subsequently expand the pool of differentiating cells. The molecular mechanisms that regulate the switch from symmetric to asymmetric divisions during cortical arealization are poorly understood. We found that the transcriptional regulator COUP-TFI, described by us to be a crucial areal patterning gene during development, restricts the stem cell pool during corticogenesis. In its absence the number of cortical stem cells/progenitors dramatically increases and progenitors become capable of long-term expansion *in vitro*. We also show that COUP-TFI directly regulates Pax6 expression during symmetric and asymmetric progenitor divisions, and during the transition between neurogenesis and gliogenesis. Thus, our data suggest that the transcription factors COUP-TFI and Pax6, normally expressed in opposite gradients and required to co-ordinately control areal and laminar identity during development, co-regulate each other during critical periods of cortical expansion.
Our work also shows that COUP-TFI is maintained in neural stem cells in the two major neurogenic regions of the adult brain, the subventricular zone of the lateral ventricle (SVZ) and the subgranular zone (SGZ) of the hippocampus. To challenge its role in adult neural stem cells, we have genetically inactivated COUP-TFI in cortical progenitors, in cortical post-mitotic neurons and at post-natal stages. When COUP-TFI is inactivated in all cortical progenitors from E10.5, the dentate gyrus is reduced and displaced and the lateral ventricle is hugely enlarged in adult mice. In mouse brains in which COUP-TFI is inactivated solely in post-mitotic neurons, no alterations are detected in the hippocampus, indicating a key role for COUP-TFI in dentate gyrus granule maturation during early stages of development. Finally, absence of COUP-TFI at P3 shows an increase of the number of stem cells in the dentate gyrus, in agreement with our embryonic analysis. In summary, our results indicate that COUP-TFI is implicated in regulating particular aspects of stem cell development, and propose COUP-TFI as a novel factor required in modulating the rate of embryonic and adult neural stem cells.

Is-29: Rewiring Point-to-Point Connections in The Mammalian CNS by Neural Transplantation. Part 1: Neurogenic Capacity and Guidance Factors in The Recipient Environment

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Cell replacement is a most promising approach to rewire disrupted neural circuits after degeneration or injury. Effective repair requires that donor cells adopt desired phenotypes and integrate in a specific manner into the host network. Successful integration of new neurons into pre-existing circuits results from complex interactions, which regulate the specification and differentiation of donor elements as well as their morpho-functional incorporation in the texture of the recipient tissue. The mechanisms and the mediators underlying these processes are largely unknown. To address these issues we investigated neuronal integration in the adult cerebellum. The cerebellar network comprises a limited

number of well-established neuronal phenotypes, which are embedded in precisely patterned structures and develop according to well known spatio-temporal schedules. By taking advantage of these suitable conditions, we first examined the cell specification and neurogenic potentiality of the developing and adult cerebellum. We showed that different mechanisms regulate the specification of distinct cerebellar phenotypes. Some lineages are restricted from early developmental phases, whereas others are multipotent and adopt distinct mature identities according site/age-specific instructive cues. Neurogenic potentialities are completely suppressed in the adult cerebellum and cannot be boosted by neuronal degeneration or application of growth factors. Nevertheless, selective mechanisms allow type-specific replacement of degenerated neuron populations, such as Purkinje cells in mutant mice. Therefore, while the adult cerebellar environment provides some conducive cues for grafted cells it does not contain adequate neurogenic information to direct the specification of multipotent donors. As a consequence, successful cell replacement in the cerebellum requires prior specification of donor cells towards cerebellar phenotypes.

Is-30: Rewiring Point-to-Point Connections in The Mammalian CNS by Neural Transplantation. Part 2: Migratory Pathways and Anatomical Incorporation of New Neurons in The Recipient Circuits

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Effective CNS repair by transplantation of stem/progenitor cells requires new neurons to functionally integrate into the recipient network, i.e. migration, acquisition of phenotype, and formation of afferent/efferent connections with target cells. Successful completion of this process depends on the interplay between donor neurons and the surrounding microenvironment. Much is known about the intrinsic properties of stem/progenitor cells, less clear are the extrinsic mechanisms that regulate their interaction with host tissue. To elucidate mechanisms of cellular incorporation in the cerebellar network, we assessed the fate of transplanted Purkinje and granule neurons, asking how architectural maturation of the cerebellar cortex during embryonic, juvenile, and adult ages influences the process of engraftment. We show that both donor phenotypes efficiently navigate through the host parenchyma either along their natural migratory pathways or following unusual routes. While granule cells achieve high degrees of integration regardless of recipient age or migratory path, the navigation and final settling of Purkinje cells varies with host age, depending on the ontogenetic construction of cerebellar cortical layering and, particularly, on the maturation of granule cells. Thus, the cerebellum can be receptive to cell replacement after the end of development, even when physiological routes are no longer viable. Yet, the degree of engraftment is dependent on specific constraints imposed by the developmental stage of the cerebellar cortex, and cannot be attributed to inadequate properties of donor cells. Understanding and exploiting the mechanisms involved demonstrates the possibility of manipulating the adult environment to further improve replacement therapies in CNS repair.

Is-31: Pluripotent Stem Cell Technology for Myelin Repair

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Stem cell biology has garnered much attention due to its potential to impact human health through disease modeling and cell replacement therapy. This is especially pertinent to myelin-related disorders such as multiple sclerosis and leukodystrophies where restoration of normal oligodendrocyte function could provide an effective treatment. Progress in myelin repair has been constrained by the difficulty in generating pure populations of oligodendrocyte progenitor cells (OPCs) in sufficient quantities. Pluripotent stem cells theoretically provide an unlimited source of OPCs but significant advances are currently hindered by heterogeneous differentiation strategies that lack reproducibility. We provide a platform for the directed differentiation of pluripotent stem cells through a defined series of developmental transitions into a pure population of highly expandable OPCs in ten days. These OPCs robustly differentiate into myelinating oligodendrocytes both *in vitro* and *in vivo*. Our results demonstrate that pluripotent stem cells can provide a pure population of clinically-relevant, myelinogenic oligodendrocytes and offer a tractable platform for defining the molecular regulation of oligodendrocyte development, drug screening, and potential cell-based remyelinating therapies.

Is-32: Transcription Factor-Mediated Reprogramming of Fibroblasts to Expandable, Myelinogenic Oligodendrocyte Progenitor Cells

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Remyelination remains an elusive goal for treating disorders such as multiple sclerosis and leukodystrophies. A number of recent studies have laid the foundation and catalyzed current enthusiasm for the use of line-

age conversion as a potential regenerative therapy for many neurological disorders. However, transplantation and functional integration of fully mature cells such as neurons and oligodendrocytes has clear limitations, therefore expandable somatic progenitors are a sought after target for cell-based therapies. We show the ability to directly convert mouse embryonic and lung fibroblasts to 'induced' oligodendrocyte progenitor cells (iOPCs) using a defined set of transcription factors. The iOPCs exhibit morphological and molecular features consistent with bona fide OPCs and can be expanded *in vitro* for multiple passages while retaining the ability to differentiate into 'induced' oligodendrocytes (iOLs) capable of ensheathing and myelinating axons both *in vitro* and *in vivo*. Our data demonstrate that the lineage conversion of somatic cells to expandable iOPCs may provide a novel strategy to study the molecular control of oligodendrocyte lineage identity and potentially for autologous remyelinating therapies in the future.

Is-33: Spermatogonial Stem Cell: Their Potency

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Since their discovery, embryonic stem (ES) cells have been proposed as a very promising source of cells for the rescue of patients suffering from diseases of specific cell loss or cell damage such as Alzheimer's disease, Parkinson's disease, spinal cord injury, diabetes and cardiac failure. However, a major drawback is that these cells are derived from embryos most likely not genetically identical to the affected patients. Therefore constituent immune suppression will be necessary when applied in regenerative medicine. Moreover, generation of human ES cells require the destruction of human preimplantation embryos, which is considered ethically unacceptable by many countries. As a result of these drawbacks, researchers have focused on finding alternative sources of pluripotent cells in the adult human body. In 2003, spontaneous *in vitro* transition of spermatogonia stem cells into the pluripotent cell state has been reported using mouse testis tissue. As long term culture and propagation of spermatogonial stem cells has been established, generation of sufficient cells for regenerative therapy could be generated. In an effort to establish an analogous source of human patient-specific pluripotent stem cells, translation of the research on transition of spermatogonial stem cells from the mouse models to the human situation has been attempted. Whether testes derived ES-cell-like cells from humans are equally potent as human ES cells is questionable.

Is-34: Spermatogonial Stem Cells: from Biology to Clinic

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Survival rates for patients with pediatric cancer have continuously improved over the past decades, due to the development of more advanced cancer therapies. However, chemotherapeutic drugs not only effectively kill cancer cells, but also destroy spermatogonial stem cells (SSCs), the stem cells of spermatogenesis. As a consequence, male sterility is a relatively common side effect of cancer treatment. A potential future clinical application to preserve fertility in these boys with cancer is to cryopreserve a small testis biopsy prior to cancer treatment, isolate and propagate the SSCs from this biopsy and autotransplant these cells after cure for cancer. Studies in animal models have provided evidence that this method might indeed preserve the fertility. In 1994 transplantation of testicular cells from a recipient mouse to the testis of a donor mouse was described for the first time. The SSCs from the recipient mouse were able to migrate to the basal membrane of the seminiferous tubules and colonize the testis of the donor mouse, giving full spermatogenesis of which sperm was capable of fertilizing eggs and producing healthy offspring. Later on, SSC transplantation has been described for many animal species indicating that this method is no species specific. Xenotransplantation of SSCs of various species into the testis of a donor mouse has been developed as the ultimate tool to recognize SSCs. Using the transplantation assay as a read out, SSC characteristics could be studied in more detail, resulting in the establishment of a long term culture of mouse SSCs that after transplantation could produce sperm to generate offspring. These techniques are now translated to the human situation to establish a clinical application of SSC autotransplantation for preserving male sterility in young boys diagnosed

Is-35: Role of Endothelial Progenitor Cells (EPCs) in the Pathogenesis of Diabetic Retinopathy

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Objective: Proliferative diabetic retinopathy (PDR) is characterized by the formation of abnormal new vessels in the retina due to both the self-expansion of vascular endothelium and the contribution of bone marrow-derived endothelial progenitor cells (EPC). In line with this evidence, we previously observed that circulating EPC from patients with type 1 diabetes and untreated PDR had a greater clonogenic capacity than EPC from patients with no signs of retinopathy. Whether an abnormal number or function of EPCs also characterize earlier stages of diabetic retinopathy and, consequently, whether EPCs may have a role in the pathogenesis of

this complication of diabetes, is presently unknown.
Materials and Methods: In type 1 diabetic patients with (A) < 20 years of diabetes with signs of non-proliferative retinopathy (n=19), (B) > 25 years of diabetes without retinopathy (n=20), (C) <5 years of diabetes without retinopathy (n=19) and age-and gender-matched nondiabetic controls (n=17), we measured the number of circulating EPCs (CD45dim, CD34+,VEGFR-2+) by flow cytometry; their clonogenic potential by the Hill's assay; and plasma concentrations by ELISA of VEGF and SDF-1, the two cytokines known to mobilize EPC. All measurements were performed when plasma glucose levels were between 70 and 200 mg/dl.
Results: The clonogenic potential of EPCs was significantly increased in group A when compared to group B ($p<0.0007$) and to non diabetic controls ($p<0.002$). Group C did not significantly differ from groups A and B possibly suggesting that, along with time, some of its patients will probably move to group A and some to group B. In contrast, the number of circulating EPCs and the cytokines plasma levels were similar in the four groups. Interestingly, platelet-associated VEGF was significantly increased in group B when compared to groups A ($P=0.006$) and C ($p=0.0005$), possibly suggesting a protective role of this parameter on the retina. The differences in clonogenic potential between the diabetic groups were not explained by different glycemic control (blood glucose and HbA1c).
Conclusion: Increased clonogenic potential of EPCs characterizes patients with type 1 diabetes and non-proliferative retinopathy. This abnormality is therefore not restricted only to the proliferative stage of this complication, but seems to parallel its development. Whether the increased clonogenic potential of EPCs is directly involved in the pathogenesis of diabetic nephropathy and, consequently, whether the progression of this complication could be controlled by normalizing this abnormality, remains to be investigated by further studies.

Is-36: Identification and Characterization of Dedifferentiated Podocytes in The Normal Renal Glomerulus

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Objective: A number of studies have demonstrated that the renal podocyte expresses the insulin receptor and has therefore to be considered a new target of this hormone. Accordingly, podocyte-specific insulin receptor knockout mice were shown to undergo effacement of the podocyte's foot processes and to develop human-like diabetic nephropathy. As a consequence of these findings, insulin resistance at podocyte level was suggested as a possible explanation for the development of diabetic nephropathy.
Recent reports have shown that podocytes share a number of specific proteins and functions with mature neurons that, just like podocytes, are also an established target of insulin. Neuron differentiation and functionality inside the adult brain seems to be maintained by the secretion of local insulin performed by immature neurons rather by systemic insulin levels. Whether local secretion of insulin by immature podocytes may also characterize the renal glomerulus is presently unknown.
Materials and Methods: Immature podocytes were searched inside normal human glomeruli of frozen renal sections looking for cells that, along with the expression of podocyte markers, were also able to secrete insulin (as demonstrated for immature neurons) and to synthesize embryonic transcription factors (an established feature of reprogramming cells).
Results: Clusters of insulin secreting cells were found in the glomerulus. These cells express some, but not all, podocyte markers and show cytoplasmic segregation of embryonic transcription factors. By applying the same protocol used to selectively grow in culture the immature neurons (that takes advantage of their aptitude to secrete insulin.), also immature podocytes were successfully grown from normal glomeruli, selected and characterized *in vitro*.
Conclusion: Altogether these findings suggest that, as previously demonstrated for the brain, in the glomerulus are localized clusters of immature, insulin secreting podocytes surrounded by their differentiated counterpart that, on the other hand, need insulin to maintain differentiation and functionality. Whether the effacement of podocyte's foot processes that characterizes the early phase of diabetic nephropathy is the consequence of insulin resistance at podocyte level or may result instead from the unbalance of local insulin secretion, and which impact might have the development of diabetes (both type 1 and 2) on an insulin secreting immature podocyte residing inside the glomerulus, remains to be clarified by further studies.

Is-37: *Ex Vivo* Expanded Hematopoietic Stem Cells Overcome the MHC Barrier in Allogeneic Transplantation

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Objective: The lack of understanding of the interplay between hematopoietic stem cells (HSCs) and the immune system has severely hampered the stem cell research and practice of transplantation. Major problems for allogeneic transplantation include low levels of donor engraftment and high risks of graft-versus-host

disease (GVHD). Transplantation of purified allogeneic HSCs diminishes the risk of GVHD, but results in decreased engraftment. Here we show that ex vivo expanded mouse HSCs efficiently overcame the major histocompatibility complex barrier and repopulated allogeneic recipient mice.

Materials and Methods: An 8-day expansion culture led to a 40-fold increase of the allograft ability of HSCs.

Results: Both increased numbers of HSCs and culture-induced elevation of expression of the immune inhibitor CD274 (B7-H1 or PD-L1) on the surface of HSCs contributed to the enhancement.

Conclusion: Our study indicates the great potential of utilizing ex vivo expanded HSCs for allogeneic transplantation, and suggests that the immune privilege of HSCs can be modulated.

Keywords: Hematopoietic Stem Cells, Allogeneic Transplantation, *Ex Vivo* Expansion, Immunology

Oral Presentations

Os-1: Study of The Genomic Stability of *In Vitro* Cultured Rat and Human Mesenchymal Stem Cells

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Objective: Bone-marrow mesenchymal stem cells (MSCs) are multipotent cells capable of self-renewal and differentiation into multiple cell types. Accumulated preclinical and clinical evidences indicate that MSCs are good candidates to be used in cell therapy for the treatment of many degenerative diseases. For MSCs clinical applications, an adequate number of cells are necessary so an extensive *in vitro* expansion is required. The importance of analyzing rat MSCs (rMSCs) is related to their use as a model for understanding their human MSCs (hMSCs) relatives.
Materials and Methods: We evaluated and compared the biological characteristics and the chromosomal status of rMSCs and hMSCs at several culture passages *in vitro*. We first used the conventional traditional cytogenetic techniques, in order to have the opportunity to observe even minor structural abnormalities and to identify low-degree mosaic conditions. Subsequently, a more detailed genomic analysis was conducted by array comparative genomic hybridization.
Results: We demonstrated that, rMSCs manifested a markedly aneuploid karyotype and a progressive chromosomal instability in all the passages we analyzed and that they are anything but stable during *in vitro* culture. On the contrary, hMSCs *in vitro* cultures were characterized by a normal karyotype and chromosomal stability in most of the donors and culture passages examined.
Conclusion: Our results support the idea that there is a considerable risk for long-term rMSC cultures due to their genomic instability. Despite the fact that the risk of neoplastic transformation associated with this genomic instability needs to be further addressed and considering the apparent genomic stability reported for *in vitro* cultured hMSCs, our findings underline the fact that rMSCs may not in fact be a good model for effectively exploring the full clinical therapeutic potential of hMSCs.
Keywords: Cytogenetic Analysis, Mesenchymal Stem Cells, Chromosomal Status

Os-2: Nanomechanotransduction of Human Mesenchymal Stem Cell

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Objective: Mechanical stimulation of human mesenchymal stem cells has demonstrated changes in many cell behaviours through mechanotransductive pathways. In this research we are looking for human mesenchymal stem cell responses to nanoscale mechanical vibrations in Z-axis.
Materials and Methods: A simple protocol for the stimulation of cells in nanoscale Z-axis has been developed for this project. Piezo actuator connected to the cell culture dish moves the entire surface up and down. The amount of displacement is dependent on the voltage applied. Attaching an aluminium disk to the base of the Petri dish ensures faithful transfer of the vibration to the cells. Human mesenchymal stem cells from bone marrow (PromoCell®) were seeded with 10,000 cells/dish. After 4 hours seeding and cell settlement, the cells were stimulated for 24 hours, 1 week, and 2 weeks. Experiments were performed in an incubator with optimal temperature 37°C and 5% CO2 concentration. The Petri dish was 60 mm x 15mm standard tissue culture treated polystyrene dish (52mm base diameter) from Corning Incorporated and cell culture media used was MEM alpha modification with L-Glutamine and nucleosides from PAA laboratories (Austria) supplemented with 10% FBS and antibiotics.
Results: We observed significant responses after 1 and 2-week stimulations in cell number, cell shapes and phenotypical markers. Microarray was performed for all groups and data is currently being analysed. Cell count shows significant increase in cell growth with 2 week stimulation. However, cell surface area, cell perimeter, and arboration after 1-week stimulation showed significant increases. Immunofluorescent studies have showed significant increase in osteocalcin production after stimulation.
Conclusion: Cell morphology changed to become more polygonal and increased expression of the osteoblast marker osteocalcin was noted suggesting nanoscale mechanostimulation might be used to stimulate osteogenesis.
Keywords: Mesenchymal Stem Cell, Mechanical Stimulation, Osteogenesis, Piezo Actuator

Os-3: c-Myb is a Key Regulator of Stem Cell Function in Multiple Epithelial Compartments

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Objective: The regulation of tissue development and subsequent tissue homeostasis is partly achieved through the orchestration of transcription factor expression and gene activation within stem and progenitor cell populations. Transcription factor c-Myb has emerged as

a key regulator of stem/progenitor cells within multiple tissue compartments such as the gastrointestinal tract (GI), mammary gland, adult brain and epidermis. Our objective has to understand how Myb co-operates with other transcription factors and their consequent regulation of stem cell genes.

Materials and Methods: We have achieved this be combining *in vitro* assays, radiation challenges and a unique set of mouse models exploitation of global and tissue specific knock-outs and hypomorphic mutants. Our focus has been on stem cell gene expression and the effects on tissue homeostasis and tumorigenesis.

Results: In the case of the mammary gland we have now shown that Myb is required for normal and timely development and its ablation can eliminate mammary tumor formation (1). In the brain adult neurogenesis is profoundly blocked (2) while in the GI crypt development is impeded leading initially biased goblet cell development and then crypt loss (3). We then focused on the GI where the Wnt target gene, Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5) is expressed. Along with it being an important GI stem cell marker it is also expressed in colon cancer cells and identifies cells capable of forming crypt-villus like structures in culture from single sorted (Lgr5+ve) cells. Based on co-incidental expression of lgr5 and c-myb in the GI, we investigated whether lgr5 is a c-Myb target gene. Using both *in vitro* and *in vivo* mouse models, we show that the proto-oncogene c-Myb in combination with β-catenin, is bound to and is a more potent regulator of the murine lgr5 promoter in the presence of activated β-catenin (4). Using an inducible c-Myb transgenic model we have further identified the regulation of CyclinE1 and Bmi1 as key GI stem cell genes that are controlled by c-Myb.

Conclusion: These data indicate that the Wnt pathway through β-catenin converge with c-Myb in regulating lgr5 expression in the GI and more broadly in other epithelial tissues with the capacity to affect tumorigenesis.

Keywords: c-Myb, Lgr5, Bmi1, CyclinE1, Tumorigenesis

Ps-1: Stable Transfection of BMSC for Using in Rat Spinal Cord Injury Model

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Objective: One of the most important basic techniques to study a specific protein in molecular biology, cloning and expression in the cells. Including gene transfer methods, non-viral methods are less expensive, easier and safer to be implemented. For gene transfer therapy is desired, a carrier shall be entitled vector. Vector plasmid, small molecules of DNA are spiral-layer. Neurotrophins are a family of proteins that induce the survival, development, and function of neurons. They belong to a class of growth factors, secreted proteins that are capable of signaling particular cells to survive, differentiate, or grow. Growth factors such as neurotrophins that promote the survival of neurons are known as neurotrophic factors. Neurotrophic factors are secreted by target tissue and act by preventing the associated neuron from initiating programmed cell death - thus allowing the neurons to survive. Neurotrophins also induce differentiation of progenitor cells, to form neurons. Although the vast majority of neurons in the mammalian brain are formed prenatally, parts of the adult brain (for example, the hippocampus) retain the ability to grow new neurons from neural stem cells,a process known as neurogenesis. Neurotrophins are chemicals that help to stimulate and control neurogenesis.
Materials and Methods: CNTF gene using PCR and cloned in plasmid pcmvsport6 after it was transformed in Escherichia coli bacteria strain DH5-alpha. The recombinant plasmid was extracted from the bacterial host and CNTF genes using enzyme ECORV, Hind III were isolated from the plasmid. The plasmid for the acceptance of cloning fragments CNTF and CNTF gene into the PEGFP plasmid was cut with the enzyme HINDIII was sub cloned. Binding reaction product in the transformed bacteria were grown in LB containing ampicillin.Recombinant plasmids using a plasmid extraction kit, and then purified from bacterial cells were transfected BMSc and the expression plasmid terminated with SDS page, western blotting was studied.
Results: Enzyme analysis showed that pcDNA had correct structure and sequencing confirmed by 100% homology of the gene with reported alpha gene in Gene Bank. In the analysis of proteins isolated from transfected cells with SDS-PAGE, approximately 45 kDa band was observed with the monoclonal antibody was confirmed by Western blot analysis.
Conclusion: Stable Transfection of BMSC suitable for using in rat spinal cord injury model.
Keywords: Transfection, Molecular Cloning, CNTF, Expression Vector

Ps-2: *In Vitro* Differentiation Bone Marrow Stem Cell Derived Neural Stem Cell into Oligodendrocyte-Like Cell

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Objective: The induction of Bone marrow stem cells in to neural and glial phenotypes was ducomented, however, the yield was low. in this investigation we tried to increase the yield of oligodendrocyte –like cells, by production of neurospheres derived from Bone marrow stem cell then neural stem cell was isolated the from neurospheres and induced in to oligodendrocyte –like cells.
Materials and Methods: In this study BMSCs of adult female rats were expanded and then was induced in to form neurospheres in the presence of B27, epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), and subsequent isolation of neural stem cells. This was followed by induction into oligodendrocyte like cells with heregulin, PDGF-AA,BFGF and T3. Which are used for transdifferentiation of neuroepithelial cells into oligodendrocyte like cells. After induction the expression profile showed O1, O4, S100 and GFAP neurofilaments. Also the expression of OCT4, B2M, MOG and PDGFR-alpha mRNA was noticed using RT-PCR technique.
Results: The Immunohistochemical and RT-PCR studies showed expression the using fibronectin, CD44, CD90, CD45 and Oct-4 and B2M which indicated that following 4th passage, a 94% percentage of cultured cells were BMSCs.The yield of oligodendrocyte like cells was about 82% using O1,O4 to characterized the oligodendrocyte –like cell.The expression of MOG and PDGFR-ALPHA in oligodendrocyte like cells, 6 day after treatment.
Conclusion: We demonstrated that neurospheres derived BMSCs could be differentiated into neural stem cells then oligodendrocyte like cells in the presence of heregulin, bFGF,PDGF and T3.
Keywords: Neural Stem Cell, Bone Marrow Stem Cell, Oligodendrocyte-Like Cell

Ps-3: Transdifferentiation of The Adipose Tissue-Derived Stem Cells into Neuron-Like Cells Expressing Neurotrophins by Selegiline

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Objective: Adult stem cells (ASC) are undifferentiated cells found throughout the body. These cells are promising tools for cell replacement therapy in neurodegenerative disease. Adipose tissue is the most abundant and accessible source of ASC. This study was conducted to evaluate effect of selegiline on differentiation of adipose-derived stem cells (ADSC) into functional neuron-like cells (NLC), and also level of the neurotrophin expression in differentiated cells.
Materials and Methods: ADSC were transdifferentiated into NLC using selegiline where CD90, CD49d, CD31, CD106 and CD45 were used as markers for ADSC identification. Lipogenic and osteogenic differentiation of ADSC were used to characterize the ADSC. ADSC were treated with selegiline at different concentrations (from 10^{-6} to 10^{-11} mM) and time points (3, 6, 12, 24 and 48 hours). Percentage of viable cells, nestin and neurofilament 68 (NF-68) immunoreactive cells were used as markers for differentiation. The optimal dose for neurotrophin expressions in differentiating cells was evaluated using reverse transcriptase-PCR. NLC function was evaluated by loading and unloading with FM1-43 dye.
Results: ADSC were immunoreactive to CD90 (95.67 ± 2.26), CD49d (71.52 ± 6.64) and CD31 (0.6 ± 0.86), but no immunoreactivity was detected for CD106 and CD45. The results of neural differentiation showed the highest percentage of nestin and NF-68 positive cells at 10-9 mM concentration of selegiline (exposed for 24 hours). The differentiated cells expressed synapsin and neurotrophin genes except brain-derived neurotrophic factor.
Conclusion: ADSC can be an alternative source in cell-based therapy for neurodegenerative diseases using selegiline to induce ADSC differentiation to neuronal lineage.
Keywords: Selegiline, Neurotrophin, Motoeuron, Transdifferentiation

Ps-4: Spinal Cord Remyelination by NSCs Transplantation

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Objective: Demyelination of CNS axons occurs in a number of pathological conditions, including multiple sclerosis and spinal cord injury. The demyelination can be repaired by cell therapy in both humans and rodents, even within the CNS, remyelination can be achieved by neural stem cells (NSCs) implantation.
Materials and Methods: In this study,in order to test whether the transplantation of NSCs affect remyelination efficiency in rat demyelination model or not, first focal demyelinating lesions were done in the adult rat spinal cord with ethidium bromide then we prepared NSCs derived bone marrow stromal cells and transplanted them to rat demyelination model.
Results: These results indicate that NSCs are capable of differentiation to myelinating cells which eliciting remyelination and have invaluable applications in treatment of neurodegenerative diseases such as spinal cord injury.
Conclusion: BMSCs were efficiently induced into neurospheres and the NSCs were prepared and the result of transplantation indicated that NSCs are capable of differentiation to myelinating cells which eliciting remyelination.
Keywords: Neural Stem Cell, Bone Marrow Stromal Cell

Ps-5: The Influence of 'Imāmī Jurisprudential Principles on Treatment Application of Stem Cells

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Objective: The prominent progresses of the medicine during the recent decades especially on bioethics and producing stem cells have led to some hopes and fears among human lives. Since the process of producing such cells is based on the destruction of the embryo, it has provoked a number of questions worldwide, and caused various scientific, religious, and ethical challenges. Scholars, on one hand, mark it as a problem for the humanism dignity and greatness and, on the other, some concern the genetic somatic researches as great discovers in the genetic sciences, but also believe that it is a symbol of divine infinite capacity of humankind which serves human life. Without any doubt, among Abrahamic religions, Islam delivers the most comprehensive and complete definition of growing sperm, embryo, and embryonic human rights. Presenting the scientific and ethical related issues, the present study aims to propose it on the basis of 'Imāmī jurisprudential principles and declares its influence on the ethical dignity of embryo.
Materials and Methods: Basic, desk, analytical
Results: 1. On the base of the juristic presumption of ibāha and the intellectual rule of "taslīt" (people are dominant over their properties and bodies) in the theoretic principles of 'Imāmīyah, cloning is not beyond

man's power and control and, as the first principle, its lawfulness is proven. 2. Those evidences which are given to prove the prohibition of abortion don't forbid the extra embryos in cloning, for the first embryonic stage, that law marks as the start point of abortion and paying blood money, is when embryo is placed at womb, while in cloning the sperm (nutfa) is destructed before settling in womb. 3. The use of this technique for the purpose of curing diseases not only is not breaking the humanism dignity but also is to prevent human life as many traditions of the Infallibles indicate that whoever favors life to a human kind, it is just like to enliven all humans.
Conclusion: 1. On the base of the juristic presumption of ibāha and the intellectual rule of "taslīt" (people are dominant over their properties and bodies) in the theoretic principles of 'Imāmīyah, cloning is not beyond man's power and control and, as the first principle, its lawfulness is proven. 2. Those evidences which are given to prove the prohibition of abortion don't forbid the extra embryos in cloning, for the first embryonic stage, that law marks as the start point of abortion and paying blood money, is when embryo is placed at womb, while in cloning the sperm (nutfa) is destructed before settling in womb. 3. The use of this technique for the purpose of curing diseases, not only is not breaking the humanism dignity but also is to prevent human life as many traditions of the Infallibles indicate that whoever favors life to a human kind, it is just like to enliven all humans.
Keywords: Stem Cells, 'Imãmī Jurisprudential, The Human State of Embryo

Ps-6: Looking into The Plausible Auto Inhibitory Function of UBA Domain in Par1b Protein: A Chief Player in Differentiation and De-differentiation Game

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Objective: Dividing behavior of cells and the way in which macromolecules are distributed through cytoplasm, is a definitive factor in determining cell fate and its totipotency or pluripotency properties. Partitioning-defective 1b (Par1b), is a serine/threonine kinase originally identified as a polarity-regulating protein in C.elegans. This protein belongs to a family of PAR (partitioning-defective) gene products that are essential for asymmetric cell division and the establishment of cell polarity. In the present study, an activating mutation was induced in this protein to see for the contradictory reported, plausible auto inhibitory role of a domain in this protein, in order to understand the structural details that would help in development of specific inhibitors for this protein.
Materials and Methods: Protein motions were studied by molecular dynamic simulation technique and use of Gromacs4.5.4 software package. The activating mutation, T208E, was induced by DeepView software. This mutation increases the kinase activity by four fold. The simulation process was carried out in a neutral environment and in the presence of water molecule, for 20 nanoseconds. The observed motions of the protein were then analyzed with a focus on possible auto-inhibitory role of UBA domain of the protein.
Results: As was expected, the protein structure moved toward active structure. But those parts of the protein which were affected by the attracting forces of UBA domain of the protein, failed to move through this activating motion. This suggests for the auto inhibitory function of this domain.
Conclusion: Regarding this fact that the UBA domain exists only in about ten kinase proteins including Par1b, development of specific inhibitors by utilizing the functional feature of this domain can lead to a more detailed understanding of this protein role, in differentiation and de-differentiation of cells.
Keywords: Par1b, Unsymmetrical Cell Division, Pprotein Structure, MD Simulation

Ps-7: The Effect of Mechanical Loading on GATA4 Expression in Stem Cells

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Objective: Cardiomyocytes, known as cells incapable of self-renewal when injured, and therefore heart-related problems, have recently attracted a great deal of attention in regenerative medicine. But what has become more important in cell therapy, is seeking for approaches resulting in more functional cells and therefore, more effective treatments. One of these methods, whose idea has been inspired from the physical environment that the tissues deal with in the body, is mechanotransduction. This study has focused on the comparison of GATA4 (cardiac-specific transcription factor gene) expression between the rabbit mesenchymal stem cells subjected to equiaxial cyclic stretch and the ones not treated mechanically.
Materials and Methods: After aspiration of bone marrow from the iliac crest of male rabbits, percoll separation technique was used to extract mesenchymal stem cells. The isolated cells were characterized using flow-

cytometry method. Their multi-potency was explored by directing them towards adipogenic, osteogenic and chondrogenic differentiation. For cardiomyogenic differentiation, the stem cells seeded on collagen-coated membranes were studied in four groups including undifferentiated (negative control), chemically, mechanically and chemically-mechanically treated cells. Rabbit cardiomyocytes were taken as positive control. After four days, GATA4 expression in each group was quantified using Taqman based Real Time-PCR method. HPRT was taken as housekeeping gene.
Results: The results obtained from flowcytometry and multipotency tests validated the extraction process and the stem cells' ability to differentiate, respectively. The Real Time PCR data showed that expression of GATA4 in cells subjected to mechanical-chemical and mechanical treatment was more than its expression in the other two groups.
Conclusion: Our results suggest that mechanical loading as a single differentiation factor, affects the differentiation of mesenchymal stem cells into cardiomyocytes. Combination of Chemical factors with mechanical loading may increase the differentiation of stem cells into cardiomyocytes and produce functional cardiomyocytes in engineered tissues.
Keywords: Mesenchymal Stem Cells, Mechanotransduction, GATA4

Ps-8: Role of Menstrual Blood Cells in Treatment of Stroke

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Objective: The progressive aging of the population has highlighted the importance of discovering therapeutic alternatives for diseases of high incidence and disability, such as stroke. The only approved therapy for stroke is tPA, strongly limited by the short therapeutic window and hemorrhagic complications, therefore excluding most patients from its benefits.
Materials and Methods: In this review article, electronic searches were undertaken in PubMed, Scholar google and up to date, since 2009.
Results: Menstrual blood is a source of stem cells with potential relevance for the treatment of stroke that is recurring and readily accessible. Immunocytochemical assays of cultured menstrual blood reveal that they express embryonic-like stem cell phenotypic markers (Oct4, SSEA, Nanog), and when grown in appropriate conditioned media, express neuronal phenotypic markers (Nestin, MAP2). Migration to the infarct site, modulation of the inflammatory reaction, secretion of neurotrophic factors, and possible differentiation warrant these cells as therapeutic tools. Transplantation of menstrual blood-derived stem cells, either intracerebrally or intravenously and without immunosuppression, after experimentally induced ischemic stroke in adult rats also significantly reduced behavioral and histological impairments compared to vehicle-infused rats.
Conclusion: Menstrual blood-derived cells exemplify a source of "individually tailored" donor cells that completely match the transplant recipient, at least in women. Neurostructural and behavioral benefits afforded by transplanted menstrual blood-derived cells support their use as a stem cell source for cell therapy in stroke. Menstrual stem cells are a novel cell population that may be routinely and safely isolated to provide a renewable source of stem cells from child-bearing women.
Keywords: Stem Cell, Menstrual, Stroke

Ps-9: The Bone Marrow Mesenchymal Stem Cells Co-culture Effects on *In Vitro* Maturation of Ovine Oocytes

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Objective: Mesenchymal stem cell (MSC) secreted a variety of cytokines and growth factors in addition to self-renewal and multiple forms of differentiation. Some of these secreted bioactive factors could improve meiotic maturation *in vitro* and subsequent embryo development potential. The aim of the present study was determine weather *in vitro* maturation (IVM) of ovine oocyte improve by co-culture with MSCs.
Materials and Methods: Fresh bone marrow aspirates were obtained from the femur of adult sheep then MSCs cultured in DMEM media. Ovine ovaries were used for aspiration of follicules collected oocyte. Immature ovine oocytes were maturated in four different medias as MSCs co-culture media, epithelial oviduct cell (OCM) co-culture media, PVA media, TCM199+FCS media. Nuclear maturation determined with DAPI staining after 24 hours.
Results: There was significant difference among groups at nuclear maturation oocyte. nuclear maturation rate was higher for MSCs media and TCM199+FCS compared with OCM media and IVP media was the lowest maturation rate. The result of expansion cumulus showed, TCM199+FCS media was most expanssion cumulus rate than MSCs media, OCM media, IVP media (respectively).
Conclusion: *In vitro* maturation (IVM) of ovine oocyte improved by co-culture with MSCs and TCM199+FCS.
Keywords: IVM, Oocytes, MSCs, Co-Culture

Ps-10: Assessing Various Antigen Panels to Enumerate Circulating Angiogenic Cells

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Objective: Neovascularization is a process recently proven to be seen in adults and probably is mediated by endothelial progenitor cells (EPCs). "Circulating angiogenic cells (CACs)" as a subtype of EPCs are defined as CD34+CD133+CD309+CD45+ cells. Many studies have been performed to analysis the correlation of EPC subtypes with different cardiovascular diseases. We aimed this study to find whether there is any correlated antigen panel to enumerate these cells or not?
Materials and Methods: The absolute number of CACs was measured in the peripheral blood of 54 volunteers using quantitative flowcytometry using CD34-FITC, CD133-PE, CD309-APC and CD45-PerCP. Ten parameters including various combinations of cell surface antigen panels were assessed. The correlations between parameters were evaluated by spearman correlation study.
Results: There is a very strong correlation between CD34 + CD133 + CD309 + CD45 + and CD34 + CD133 + CD309 + numbers (correlation coefficient = 0.998 and p <0.001). Number of CACs had strong correlation with the number of CD133+ cells and CD133 + CD309 + cells.
Conclusion: Here we have shown that a three color analysis of CACs is absolutely performable and a two color study is also possible but needs further studies. This may help making the researches easier and also helping reducing the costs.
Keywords: Endothelial Progenitor Cell, Circulating Angiogenic Cells, CAC

Ps-11: Assessing The Correlation in The Number of Endothelial Progenitor Cell Subtypes with Each Other

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Objective: Neovascularization is a process recently proven to be seen in adults and probably is mediated by endothelial progenitor cells (EPCs). Several types of cells are under the umbrella of EPCs: "endothelial colony forming cells (ECFCs)", "colony forming unit endothelial cells (CFU-ECs)" and "circulating angiogenic cells (CACs)". Many studies have been performed to analysis the correlation of EPC subtypes with different cardiovascular diseases. We aimed this study to find whether the frequency of these cells show any correlations with each other or not?
Materials and Methods: The absolute number of different subtypes of EPC was measured in the peripheral blood of 54 volunteers using quantitative flowcytometry and culturing techniques. The correlations between parameters were evaluated by spearman correlation study.
Results: The number of CACs and CFU-EC number had an inverse non significant correlation in (P value=0.446). Among the putative flowcytometric enumerated ECFCs, and other subtypes of EPC no correlation was noticed either.
Conclusion: Here we have shown that analysis of different subtypes of EPCs together in one study can explain controversies found in the literature regarding the frequency of EPCs. Although each of EPC subtypes have been shown to have correlations with cardiovascular diseases but the absolute numbers of subtypes varies independently.
Keywords: Endothelial Progenitor Cell, Colony Forming Unit Endothelial Cell, Endothelial Colony Forming Cell, Circulating Angiogenic Cells, Colony Forming Unit

Ps-12: Transplantation of GFP-Expressing Neuron Like Cells Drived from P19 Stem Cells into Midbrain of Chicken Embryo

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Objective: P19 cells are a line of pluripotent embryonal carcinoma stem cells able to grow continuously in serum-supplemented media. Also p19 cells are amenable to genetic manipulation. when these cells treated with deprenyl can differentiate into cell types similar to those derived from neuroectoderm. The antiparkinsonian effect of deprenyl was reported by several investigators. Because of the limited regenerative potential of adult neural tissue, stem cells may provide a means of surmounting this problem, because they can generate unlimited numbers of cells for use in tissue replacement therapies. Recent reports have shown that many kinds of stem cells can integrate into the chicken embryo and differentiate into various cell types with apparent fusion to the host chicken cells. In this investigation,

deprenyl was used to induce neuronal differentiation in GFP-transfected pluripotent P19 cells.
Materials and Methods: The cells were cultured using α-MEM medium that supplemented with 15% FBS. The optimal inducing dose of deprenyl was obtained using different concentrations of deprenyl (10-6 – 10-11 M) and the peak response was at 10-8 M. Morphologic and immunofluorescence techniques were used to evaluate the differentiation of the P19 cells, Cresyl violet for the morphologic study, anti-synaptophysin and anti-Beta-Tubulin III antibodies for characterizing the neuronal phenotype of the cells. Then we implanted P19-derived neurons into midbrain of 68-72 hours developed chicken embryo. We used anti-synaptophysin and anti-Beta-Tubulin III and anti-GFP antibodies by using of duble-immunofluorescence techniques in chicken tissue sections to detect the implanted GFP positive P19-derived neurons and follow their migration.
Results: The results showed that deprenyl could induce dose-dependent GFP-transfected P19 differentiation into neurons. Also, GFP positive P19-derived neurons could migrate in neuronal system of chicken embryo and make synapse with host tissue cells.
Conclusion: According to our finding the combined deprenyl and stem cell therapy can be considered to improve deficits in neurodegenerative diseases.
Keywords: Embryonal Carcinoma Stem Cells, Neuronal Differentiation, Deprenyl, Cell Implantation, Chicken Embryo

Ps-13: Effect of Peptidoglican-Lipopolysaccharides, A Ligand of TLR4 Primed MSCs on The Production NO in The MSCs

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Objective: Mesenchyme stem cells (MSCs) have potent regulatory effects on immune and inflammatory responses. The MSC-mediated immunosuppression mainly acts through the secretion of soluble molecules such as NO. NO plays a major role in immune regulation and has been shown to affect TCR signaling, cytokine receptor expression, and the phenotype of T cells. Murine MSC (muMSCs) TLR activation is essential for inducing the immune response and enhancing adaptive immunity against pathogens. We have investigated the effect of peptidoglycan-lipopolysaccharides (LPS), a ligand of TLR4, priming MSCs on the production NO.
Materials and Methods: MSCs were isolated from bone-marrow of male mice and cultured *in vitro*. MSCs were nontreatment (control) or treatment with a TLR2, 4-agonist (peptidoglycan-LPS, 10 ng/ml) for different times (1 hour and 12 hours) and assessed NO with Graise test.
Results: Results: Data showed that short term exposure (1 hour) with agonist TLR2, 4 on MSCs significantly increases on the production No as compared to the control group.
Conclusion: Findings suggest that the different-Time of MSCs exposure to TLR2, 4 agonist, differently affected NO production of MSCs. The results can be applied potently for more successful MSC-based therapy programs.
Keywords: TLR, NO, MSCs

Ps-14: Treatment of Mesenchymal Stem Cells (MSCs) with Peptidoglycan-LPS As TLR2,4 Agonist and Zymozan TLR2,6 As Augments Apoptose in Activated T Cells Time Dependently

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Objective: Adult bone marrow-derived mesenchymal stem cells (MSCs) are under study as therapeutic delivery agents that assist in the repair of damaged tissues. MSCs are recruited to sites of stress or inflammation to fulfill their repair function. The differential expression of TLRs on MSCs might contribute to the discrete changes in the function of MSCs. MSCs have been shown to suppress the activity of a broad range of immune cells, including T cells. In the present study invested the coestimolatory effect of peptidoglycan-lipopolysacarid as TLR2, 4 agonist and zymozan as TLR2/6 on apoptosis induction in activated T cells by mouse mesenchymal stem cells (MSCs).
Materials and Methods: MSCs were isolated from bone-marrow of mice and treated with peptidoglycan-LPS (10 ng/ml) as TLR2/4-agonist and zymozan (25 µg/ ml) as TLR2/6 agonist for different times (1 hour and 12 hours). Treated cells were co-cultured with PHA-activated splenic mononuclear cells (MNCs) for 72 hours at 37°C in a humidified 5% CO_2. After 72 hours, the percentage of apoptosis in activated T cells was assessed by anti-CD3PE, Acridin-Orang/PI staining in flow cytometry.
Results: We found that short term exposure (1h) of MSCs to TLR2, 4 agonist (peptidoglycan-LPS) and TLR2, 6 agonist (zymozan) con significantly increase of apoptosis in activated T cells in comparison to control group.
Conclusion: Our findings suggested that different exposure terms of MSCs to combination TLR2/4 agonist,

and TLR2/6 agonist differently affected apoptotic activity of MSCs against activated T cells; so, the results can be applied potently for more successful MSC-based therapy programs.
Keywords: TLR, MSC, T Cell

Ps-15: Infection of Retinal Pigment Epithelium Cells by Lentiviral Vector Containing SOX2 Gene

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Objective: Retina is light sensitive tissue in the inner surface of the eye. It includes muller cells, photo receptors, amacrine cells, ganglion cells, bipolar cells, horizontal and RPE cells. Macular degeneration and retinitis pigmentosa are some examples of retinal diseases which affect neural retinal cells. Retinal pigment epithelium (RPE) is a pigmented layer in retina that in specific culture conditions can dedifferentiate to neural progenitor cells. This capability of RPE cells makes them as a potential source of progenitor cells in cell therapy purposes SOX2 is a transcription facto and an important marker for neural progenitors and stem cells in CNS in vertebrate. By expression of SOX2, neural progenitor stem cells characteristics are retained. The purpose of this study is to dedifferentiate RPE cells to neural progenitor cells by SOX2 over expression.
Materials and Methods: RPE cells were isolated from human eye globes and cultured in DMEM/Ham's F-12 supplemented with 20% FBS. They were subcultured in DMEM/Ham's F-12 with 10% FBS in subsequent passages and finally were characterized by ICC method for RPE65, as a marker of RPE cells. Coding region of SOX2 gene was synthesized and cloned into PCR2.1 vector, then by BamHI and XhoI restriction enzymes, SOX2 gene was digested and subc loned into pLEX-IRES- Pur Lentiviral vector. Accuracy of recombinant construct was approved by PCR, digestion and sequencing. Recombinant construct with helper vectors (PMD2G, PsPAX) were transfected to HEK293T using calcium phosphate method and the media containing virus's particles was collected from infected HEK293T cultures. Subsequently RPE cells were infected with virus particles and as a control for transfection and infection, a Plex-GFP vector was used.
Results: Transfected HEKT293 and infected RPE cells showed strong expression of GFP.
Conclusion: Successful infection of RPE cells by desired vectors made a promise for following SOX2 gene over expression and dedifferentiation of RPE cells to neural progenitor cells.
Keywords: Retinal Pigment Epithelium Cells, Human SOX2 Gene

Ps-16: Glucocorticoids Inhibit Cell Death Induced by Doxorubicin in Cardiomyocytes

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Objective: Transgenic embryonic stem cell-derived beating cardiomyocytes may be considered as a proper model for real assessment pharmacological and toxicological effects of drugs *in vitro*. In this model, We report here that glucocorticoids (GCs) such as dexamethasone (Dex) protect cardiomyocytes from apoptotic cell death induced by doxorubicin (Dox); in addition physiological functions such as heart beat, cardiac gene expression and cell survival are assessed.
Materials and Methods: In this research, Mouse transgenic ESCs line RB1α-MHC differentiated cardiomyocytes (CMs) were purified under puromycin (pur) treatment. The percentage of purified CMs were assessed by Flow Cytometry and in morphological study, cardiomyocytes purity was proved by primary antibody against α-MHC in immunostaining. Beating cardiomyocytes were treated with different concentrations of doxorubicin (Dox), dexamethasone (Dex: 10 μM) or combinations of them. Percentage of beating cardiomyocytes, quantitative Real-Time polymerase chain reaction (qRT-PCR) for cardiac gene expression (α-actin, α-SMA and β-MHC), MTS assay for cell survival were evaluated in each group. Cardiomyocytes stained positive for cardiac α-MHC and glucocorticoid receptor.
Results: Dox cardiotoxicity on mouse transgenic embryonic stem cell-derived beating cardiomyocytes can be ameliorated by treatment with Dex when Dex administrated 24 hours before Dox. The effect of Dex appears to be mediated via glucocorticoid receptors. Dex pretreatment increases cardiomyocyte gene expression and cell surveillance in Dox-treated groups, significantly. Interestingly, caspase-3 activity was markedly decreased in Dex-pretreated groups.
Conclusion: ESC technology offers the opportunity to develop functional models by expanding cell populations in the pluripotent state and subsequently converting them to mature fully differentiated cells. At the cellular level, Dox as an established drug remains the

focus of pre-clinical and clinical research. Therefore, by using Dox, different studies have aimed to improve the mechanisms of activity or reduction of cardiotoxicity, in addition to identifying new strategies that have higher therapeutic efficiency in cancer patients. It has been proposed that this improvement was possibly achieved by the improvement of expressions of 3cardiac genes, which included α-cardiac actin, α-SMA and β-MHC. The results were in accordance with studies that have used mature cardiomyocytes. Moreover, these cells have proved that could be used in molecular studies as an appropriate model.

Keywords: Transgenic Embryonic Stem Cell, Doxorubicin, Dexamethasone, Apoptosis, Cardiotoxicity

Ps-17: Evaluation of AMI Drop on Rabbit Corneal Epithelium Healing

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Objective: Amniotic membrane (AM) has several biologic benefits; anti-inflammatory, anti-scarring effects and promotion of epithelialization. In this study we are going to evaluate AM derived proteins called AMI drop on limbal stem cell proliferation and corneal healing in Rabbit model.

Materials and Methods: Proteins were extracted from human AMs which were obtained from healthy mothers at the time of cesarean section using mechanical procedure and named AMI drop. The protein concentration as well as growth factors (bFGF, EGF …) was determined by Bradford assay and ELISA respectively. Cytotoxic dose and effective dose of AMI Drop were determined using Limbal stem cells which cultured with different doses of AMI drop (0.1-1 mg/ml). The corneal epithelium mechanically was removed from 10 rabbit's eyes with 8 millimeter ring. Rabbits divided in two groups, First group received AM drop every 2 hours at right eye and second group received AM drop every 6 hours at right eye. Left eyes in both groups have chosen as control and received antibiotic and steroid every 6 hours. Complete eye exam was done every day and pictures were taken for documentation from all rabbit's eyes every morning at six post operative day. Finally rabbit's eyes sent to Iranian Eye Bank for pathologic investigation.

Results: AMI drop promoted the proliferation rate of cultured limbal stem cells at dose of 0.2-1 mg/ml without any cytotoxic effects or tumorogenic effects. Preliminary results (with 1 mg/ml of AMI drop) suggest that AM drop have all properties that we expected from Amniotic Membrane Transplantation in animal model like healing acceleration, anti-inflammatory and anti-scar formation.

Conclusion: AMI drop can be effective for ocular epithelium healing in Rabbit; and healing duration can be shorter than common treatments. However it is a novel idea which needs to do many experiments to clarify mechanism and signaling pathways.

Keywords: Amniotic Membrane, AMI Drops, Corneal Epithelium, Rabbit

Ps-18: Use of Non-Rotational Computer Controlled Suspension Bioreactor for Expansion of Non-purified Cord Blood Mononuclear Cells

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Objective: The ability to culture hematopoietic cells in readily characterizable and scalable bioreactors will aid the development of transplantation therapies. Most ex vivo experiments have reported that stirrer bioreactors can be used for expansion of hematopoietic stem cells (HSCs) from different sources. In the present study, the proliferation and differentiation characteristics of cord blood mononuclear cells (CB-MNCs) were examined in a non-rotational suspension bioreactor with fish tail mixer (test) and T-flask cultures (control).

Materials and Methods: CB MNCs were cultured in both T-flask and suspension bioreactor with 1x106cells/ml seeding density for 14 days. The bioreactor system consisted of glass vessels where cells were maintained in suspension in a homogeneous environment without the need for a stromal feeding layer, a fish tail mixer which moved in vertical axis (up and down), and a data acquisition and control system which continuously monitored pH, temperature and dissolved oxygen. The flowcytometery, colony assay and RT-PCR were performed for evaluation of hematopoietic stem cells, their colony forming potential and stemness gene expression of cultured cells.

Results: Our results determined that the total number of cells increased in the T-flask, however the bioreactor provided superior expansion of total HSCs (CD34+38-90+), expression of stemness-related genes and colony-forming cells (CFC) during 14 days of culture.

Conclusion: Although the use of a discs with up and down movement as a mixer reduces total cell numbers in a short term culture, which may be related to increased shear stress, it did not affect function of HSCs.

Keywords: Suspension Bioreactor, Vertical Mixing, Static Culture, Umbilical Cord Blood, Mononuclear

Ps-19: Overexpression Of microRNA 219 In Human Endometrial Stem Cells Contribute to Oligodendrocyte Differentiation By Reduction Of PDGFRa

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Objective: Human Endometrial-derived stem cells (EnSCs) are the abundant and easy available source for cell replacement therapy. miRNAs have a critical role in oligodendrocyte development including cell proliferation, differentiation and myelin formation. MiR-219 is necessary to promote oligodendrocyte differentiation by repressing negative regulators of oligodendrocyte differentiation including PDGFRa. Oligodendrocytes are myelinating cells in the central nervous system (CNS) that form the myelin sheath of axons to support rapid nerve conduction.
Material and Methods: The EnSCs after treating with FGF2, EGF, PDGF-AA(20ng/ml) and T3(30ng/ml) for 18 days, were infected by miR-219. 4days after infection, cells were collected and analyzed for expression PDGFRa, olig2, A2B5 by qRT-PCR and immunocytochemistry.
Results: The flow cytometric analysis showed that EnSCs were positive for CD90, CD105, OCT4, CD44, CD146 and were negative for CD31, CD34, CD133, CD45. The result showed in the infected cells, expression of PDGFRa compared with non infected and control cells significantly decreased and expression other oligodendrocyte cells markers such as Nestin, Olig2, A2B5 in the level of mRNA and protein increased in compared with control and non infected cells.
Conclusion: The EnSCs can differentiate to oligodendrocyte cells by overexpression of miR-219 and may convince to consider these cells as a unique source for cell therapy of neurodegenerative disease.
Keywords: Endometrial Stem Cell, Oligodendrocyte, Differentiation, MicroRNA, Neurodegenerative Disease

Ps-20: Differentiation of Embryonal Carcinoma Stem Cells into Insulin-Producing Cells by Using Pancreas Extract *In Vitro*

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Objective: Type I diabetes mellitus results from the autoimmune destruction of the β cells in pancreatic islets. Therefore, extensive research is going to generation of insulin-producing cells (IPCs) from stem cells. P19 embryonal carcinoma cells are multipotent and can differentiate into cell types of all three germ layers. In this study differentiation of P19 cells into IPCs by using mouse pancreas extract (MPE) was investigated
Materials and Methods: In this study embryoid bodies (EBs) resulted of P19 cells were cultured in medium containing 3% fetal bovine serum, supplemented by concentration of 50, 100, 200,300 μg/mL MPE for 7-14 days. Dithizone (DTZ) staining was used to detect IPCs derived from EBs *in vitro*. Mouse monoclonal insulin-proinsulin and monoclonal insulin receptor beta antibodies used for immunoflourescence. Insulin content from the cells and secreted insulin by differentiated cells in response to concentrations of 5/5 and 25 mM Glucose were measured using ELISA kits.
Results: DTZ-positive cells showed purple-red clusters. immunoflourescence indicated expression of Beta cell markers (insulin-proinsulin and insulin receptor beta) in these cells. The results indicated significantly increased insulin levels secretion by differentiated cells with high concentrations of MPE. These cells could respond to the increasing glucose of medium by increasing insulin secretion. These results indicated that P19 cells can serve as potential source of insulin-producing cells for transplantation therapy of type I diabetes mellitus.
Conclusion: Our study suggests that these results indicated that P19 cells can serve as potential source of insulin-producing cells for transplantation therapy of type I diabetes mellitus.
Keywords: Embryonal Carcinoma Cells, Pancreatic Extract, Insulin Producing Cells

Ps-21: Bone Marrow Derived Mesenchymal Stem Cell Transplantation in Cerebellar Degeneration Model

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Objective: In addition to its key role in complex motor function, the cerebellum is increasingly recognized to have a role in cognition. Thus, motor and cognitive deficits can be associated with cerebellar degeneration.

Materials and Methods: After unilateral lesion in cerebellum (folia VI) was caused by Quinolinic acid, CM-DiI labeled mesenchymal stem cells (MSCs), which were isolated and purified from bone marrow, were transplanted into the damaged folium. Motor function was assessed using the cylinder test, rotarod, hanging wire and beam balance during 6 weeks after transplantation. Cognitive function was assessed using the Morris water maze learning paradigm in 3 weeks after transplantation.
Results: Six weeks after transplantation surviving MSCs were detectable in QA-treated animals. The MSC-transplanted group showed markedly improved functional performance in spatial memory, motor learning, locomotor asymmetry, dysmetria, abnormality in neuromuscular strength and equilibrium 2–6 weeks compared with the controls. We found that cerebellar lesions produced deficits (folia VI) in motor and cognitive aspects of a spatial task.
Conclusion: The results indicate that transplantation of MSCs can significantly reduce the behavioral abnormalities of these animals during six weeks after engraftment. According to results of this assay, cell therapy by means of bone marrow derived adult stem cells promises for treatment of cerebellar diseases.
Keywords: Mesenchymal Stem Cell, Cerebellum, Cognition, Motor Function

Ps-22: Induced Expression of Indoleamine 2, 3-dioxygenase in Endometrial Mesenchymal Stem Cells

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Objective: Mesenchymal stem cells (MSCs) represent promising tools for the treatment of a wide range of disorders .In addition to their ability to differentiate into osteoblasts, adipocytes, myoblasts and neural cells, some studies have shown new light on their unique immunomodulatory properties and possible therapeutic use. Indeed, MSCs express the major kynurenine pathway (KP) enzyme, Indoleamine 2, 3-dioxygenase (IDO), in response to interferon gamma (IFN-γ), and thus can inhibit T cell proliferation and modulate immune systems. The human endometrium is a dynamic tissue. Regeneration follows parturition and extensive resection and occurs in postmenopausal women taking estrogen replacement therapy. It is likely that adult stem cells are responsible for this capacity. This study was aimed to evaluate the potential role of endometrial mesenchymal stem cells (EMSCs) on immune-suppression/modulation.
Materials and Methods: EMSC cultures were seeded into 96-well for immunocytochemistry. Subconfluent cultures were treated with different doses of recombinant human IFN-γ. The cells were rinsed and fixed with 25% glutaraldehyde and washed twice in PBS(phosphate buffer saline). Following the cells were treated with 4% bovine serum albumin (BSA) and washed. Cells were then incubated with primary antibody against IDO and then were incubated with secondary antibody and then washed with PBS. TMB and Stop solution were added and absorption was read at 450 nm.
Results: Preliminary results of immunocytochemistry showed the increasing amount of IDO protein expression following IFN- γ treatment.
Conclusion: Obtained results show IDO expression in INF-gamma-treated EMSCs could be subjective that may point to immunosuppressive effect of EMSCs, which need to complementary evaluations.
Keywords: Endometrial Mesenchymal Stem Cells, IDO, Expression

Ps-23: Mesenchymal Stem Cells and Homing Concerning Breast Tumor

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Objective: MSCs are multipotent adult stem cells with the ability to home in on sites of injury. They can be used as a potential vehicle for gene and cell therapy. Lack of immunogenicity and immunosuppressive phenotypes lead to expanded investigation of their homing in on tumors.
Materials and Methods: Balb/c mice were implanted with adenocarcinoma of breast, making a model of breast cancer. GFP (green fluorescent protein) expressing mesenchymal stem cells were cultured and injected to the tail vein of all breast cancer balb/c mice models.Three mice were sacrificed after 4, 12 and 15 days each. Samples of liver, lung, spleen and bone marrow were assayed by flowcytometry, comparing with the control group.
Results: Flowcytometry analysis showed that the concentration of MSC in lung tissue was higher than the other tissues. Minute amount of cells were seen in

spleen and bone marrow tissues.
Conclusion: Increased tendency of MSCs to the lung tissue may have stemmed from entrapment of these cells in this tissue.Increasing MSC number may compensate the lung entrapment. Moreover using more sensitive methods may help to detect probable implanted cells.
Keywords: Breast Cancer, Homing, Green Fluorescent Protein

Ps-24: Potential Involvement of hsa-miR-133b in Cardiomyocytes Differentiation and Proliferation

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Objective: In the heart of humans and other mammals, low renewal capacity is unable to restore heart function after any significant injury. Recent studies have shown that some microRNAs (miRNAs) inhibit cardiomyocytes proliferation by repressing the expression of multiple cell cycle regulators. Some of well-known cardiac muscle transcriptional networks, consisting of SRF/myocardin are responsible for the expression of these miRNA genes. In according to other literatures, miR-1 -2/133a-1 and miR-206/133b are two of these miRNA clusters which are expressed in cardiac and skeletal muscle, respectively. In contrast to other studies, we showed the differential expression levels of hsa-miR-133b during cardiac differentiation. Also, miR-133b differs from miR-133a by only two nucleotides at the 3' terminus. The sequence similarity of these miRNAs suggests that they share the same or similar regulatory mRNA targets. These findings could award us to suppose the potential roles for miR-133b in cardiovascular system, as well.
Materials and Methods: The human cardiac progenitor cells (c-Kit+ progenitor cells) were prepared from Royan Stem Cell Bank (RSCB), cultured and differentiated into cardiomyocytes. The differentiation into cardiomyocytes was confirmed by ICC (Immunocytochemistry) test for cardiac troponinT and real-time PCR for some early cardiac marker and sarcomeric genes. The expression profiles of hsa-miR-133b and some of its target genes including of SRF, CCND2, TGFBR1, TGFBR2 and TGFBR3 were analyzed during the processes of differentiation.
Results: Real time PCR data showed the increasing levels of hsa-miR-133b during cardiac differentiation stages. The expression levels of this miRNA in 3-4 weeks after the first differentiation induction was about five times higher than that of early stage ($p<0.05$). In terminal differentiated cardiomyocytes, the transcriptional level of this miRNA was decreased ($p<0.05$). In addition, the expression profiling of upper mentioned target genes during cardiomyocytes differentiation showed the reverse pattern of hsa-miR-133b expression profile.
Conclusion: In the heart, SRF binds the enhancer regions of the miR-1/miR-133 cluster and regulates their expression in cardiomyocytes. In this view, overexpression of miR-133b might blocks cardiomyocytes proliferation and differentiation with direct targeting of SRF (in a negative feed-back loop), CCND2, TGFBR1, TGFBR2 and TGFBR3. All together, our data award us into the regulatory networks of related genes that are involved in cardiomyocytes differentiation and proliferation.
Keywords: c-Kit+ Progenitor Cells, Differentiation, Cardiomyocytes, Proliferation, hsa-miR-133b

Ps-25: Neural Transdifferentiation of Synovium-derived Mesenchymal Stem Cells

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Objective: Synovium- derived mesenchymal stem cell (SM-MSCs) are a fascinating source for regenerative medicine because they can be harvested in a less invasive manner and are easily isolated and expanded. This study designed to characterization SM-MSCs and investigation of their neural differentation.
Materials and Methods: Human SMs were enzymatically obtained from 15 knee joints with ACL (anterior cruciate ligament) injury and baker cysts around the time of reconstruction surgery and were expanded in monolayer with serial passages. SM was plated, cultured and examined for colony-forming number, *in vitro* differentiation. Then, SMSCs induced *in vitro* by β-Mercaptoethanol (BME) and Retinoic acid (RA). Alizarin red oil red O staining and RT-PCR were done to investigate stemness property of cells. RT-PCR analysis was done to prove mRNA expression for neural marker NFM (neurofilament- medium) and early neuronal markers Vimentin.
Results: Following neural induction, SMSCs were differentiated in to various types of neural cells including neurons and glia *in vitro*. RT-PCR analysis demonstrated that mRNA encoding for NFM and Vimentin, were expressed.
Conclusion: Our study suggest that synovium tissue, which is discarded in most knee operations, can be used for cell therapy and tissue engineering protocols because it is easily regenerated after harvesting and readily available for clinical applications. Induction of SMSCs by different inducers RA and BME could highly transdifferentiate SMSCs into neuroglia and neuronal-like cells.
Keywords: Mesenchymal Stem Cells, Synovial Mem-

brane, Neural Differentiation

Ps-26: *In Vitro* Nueral Differentiation of Synovium- Derived Mesenchymal Stem Cell

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Objective: Mesenchymal stem cells are present in many human tissues and serve as a readily available source of undifferentiated cells being capable to form specific tissues like bone, cartilage, fat, muscle and tendon. MSCs can be isolated from various adult mesenchymal tissues including synovium. the aim of this study is search the ability of synovium- derived mesenchymal stem cells to differentation to acquire neuronal or glial *in vitro*.
Materials and Methods: MSCs were isolated and cultured from femoral knee joint of baker cyst and ACL(Anterior cruciate ligament)patient and identified with morphological observations and differentiation tests.Then, SMSCs isolated and induced *in vitro* by β-mercaptoethanol (BME) and retinoic acid (RA). Alizarin red oil red O staining and RT-PCR were done to investigate stemness property of cells. After differentiation, the expression of neuronal markers NF-L (neurofilament- light, or neurofilament 70 kDa) and early neuronal marker NeuroD1 were determined with RT-PCR.
Results: Morphological observations, differentiation results and RT-PCR tests revealed that our isolated cells were MSCs. RT-PCR analysis indicated that the RA and BME treated SM- MSCS expressed mRNA transcripts for neuron-specific markers neurofilament proteins (NF-L) and Neurogenic differentiation 1(NeuroD1).
Conclusion: These results indicate that human synovial membrane -derived MSCs could trans-differentiate into neural cell types by RA and BME treatment. So in spite of these results, better understanding of SM-MSCs behavior *in vitro* and *in vivo* is needed to develop strategies for therapeutic applications by these cells.
Keywords: Mesenchymal Stem Cells, Synovial Membrane, Neural Differentiation

Ps-27: Isolation and Characterization of Human Brain Cancer Stem Cells

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Objective: Cancer is a disease in which cells proliferate out of control and in malignancy lead to metastasis. Brain tumor is an example of these cancers. Timely recognition of cancer in its early stages increases the likelihood of treatment. Therefore, the identification of tumor initiating cells is accordingly important and with identifying these cells, their markers and involved signaling pathways, it would be possible to target these cells directly or indirectly and inhibit them with the least side effects for patients. As example of common markers in identifying brain cancer stem cells, CD133, Integrinα6, nestin and L1CAM (as glioma stem cell markers) and OCT4 (as a stemness marker) can be mentioned. These markers are expressed in normal cells in specific levels whereas they over express in brain cancer stem cells. They are recognized by analyzing of their expression level. Therefore in this study, we identify brain cancer stem cells, characterize and isolate them.
Materials and Methods: To achieve aims of this study we use variety of techniques. First, brain tumors were recognized and separated by surgeon; they were cultured in proper medium and produce neurosphere. Neurospheres were studied by neurosphere assay method and different passages of them were used for techniques such as immunocytochemistry, flow cytometry and Real time PCR to study of qualitative and quantitative expression of cellular markers.
Results: As a result of experiments, we concluded that increase of the listed markers related to tumor nature and stemness potential of these cells.
Conclusion: So, we can isolate and characterize cancer stem cells according to gene expression pattern and use them in research and clinical studies for improving the treatment method of CNS tumors.
Keywords: Cancer Stem Cell, Brain Tumor, Characterization, Isolation

Ps-28: Study of Gene Expression of BDNF, NGF, NT3 and NT4 Neurotrophins in Different Stages of Human Dental Pulp Stem Cells Differentiation

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Objective: Neurotrophins are growth factors which have roles in development of neural cells. Dental pulp stem cells are one of cell types that produce neurotrophins. These factors strongly affect on division, differentiation,

survival and death of neural cells. NT4, NT3, NGF and BDNF neurotrophins are produced in dental pulp stem cells; lead these cells into neural cells. Studies have been demonstrated that, level of these factors, increase in inducing dental pulp stem cells. Given the limited nature of stem cells regions and easy and reliable availability to them, we can use these cells as a suitable source of stem cells. These cells can be used in clinical and therapeutic fields, especially in neurologic and neural disorders such as Alzheimer's, Huntington and Parkinson diseases. In this study, the mRNA expression level of neurotrophins have been studied in different stages of dental pulp stem cells differentiation.

Materials and Methods: First, we designed specific primers of neurotrophins using Oligo and Beacon Designer software's. Then we used RNeasy mini kit in different stages of dental pulp stem cells differentiation to isolate cellular RNA and then cDNA was synthesized. After that, using Real time PCR, quantitative comparison of expression level of these genes was done.

Results: Results of these experiments show that neurotrophins are expressed in different stages of differentiation.

Conclusion: We can conclude that these factors are effective in cell differentiation and are useful in research and therapeutic goals.

Keywords: Dental Pulp Stem Cells, Neurotrophin, Real Time PCR

Ps-29: Study of Gene Expression of TrkA, TrkB, TrkC Tyrosine Kinase Receptors and P75 Receptor in Different Steps of Human Dental Pulp Stem Cells Differentiation

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Objective: Dental pulp stem cells are cells which can be used in treatment of neuro-degenerative diseases and injuries to the central nervous system including brain ischemia and spinal cord trauma. These cells produce variety of neurotrophic factors which are bind to TrkA, TrkB, TrkC tyrosine kinase receptors. Each neurotrophin has its special tyrosine kinase receptor, while P75 receptor is a common receptor and is able to bind to all of these factors. These receptors with their interaction with neurotrophins have roles in survival, differentiation and cell death due to stimulation of signaling pathways. In this study, we investigated the expression levels of these factors in different steps of dental pulp stem cell differentiation. Given that the increase of these receptors is as a result of increasing in neurotrophin's expression and they are involved in some neurodegenerative diseases such as, multiple sclerosis (MS) and Alzheimer's, we can use these receptors in treatment of these diseases.

Materials and Methods: We designed specific primers for each neurotrophin receptor genes. We extracted cellular RNA content from stem cells in different steps of differentiation using RNeasy mini kit and then we make their cDNA. Finally, we can evaluated changes in gene expression using Real time PCR machine.

Results: Results of this study showed that these receptors express in different levels in dental pulp stem cells.

Conclusion: So, it can be concluded that they are important in differentiation of these stem cells and we can use them in treatment of neurodegenerative diseases.

Keywords: Dental Pulp Stem Cells, Tyrosine Kinase Receptors, Neurotrophin, Real Time PCR

Ps-30: Epigenetic Role of Nuclear Actin in Oct4 Regulation of Human Embryonal Cells

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Objective: Nuclear actin is involved in regulation of transcription by recruitment of the histone modifier elements as well as chromatin remodeling factors to the regulatory regions of active genes. In recent years, more and more attention has been focused on the role of actin and actin binding proteins (ABPs) in the modulation of the subcellular localization of transcriptional regulators, through different cellular processes. In the current work, the epigenetic role of nuclear actin on transcription regulation of a pluripotency marker gene, Oct4, has been evaluated in human embryonal carcinoma cells, before and after induction of differentiation.

Materials and Methods: Using quantitative real-time PCR technique, mRNA expression levels of the marker gene Oct4 were analyzed in a human embryonal carcinoma cell line NT2/NTERA2, before retinoic acid (RA)-induced differentiation and after 3days of induction. Then, Chromatin Immunoprecipitation (ChIP) coupled with real-time PCR were performed on the cellular samples, in order to monitor the incorporation of actin protein on the regulatory region of the aforementioned gene.

Results: The results showed a significant change in incorporation of nuclear actin on the promoter region of Oct4, in accompany with down regulation of the gene after RA-induced differentiation.

Conclusion: This preliminary finding implies the dy-

namic epigenetic role of nuclear actin in cellular differentiation.
Keywords: Nuclear Actin, Epigenetic, Embryonal Cells

Ps-31: Stemness Inhibiting MiR-200 Family Target Sequence for Translational Repression of Therapeutic Gene in Cancer Stem Cell Rich MDA-MB231 Cell Line

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Objective: Metastasis of carcinomas is promoted by the activation of the embryonic (EMT) program. The (ZEB1) is a crucial inducer of EMT and metastasis in various human tumours and it inhibits expression of the microRNA 200 family by binding to the E-box or Z-box modules which are located in the promoters of miR-200 family whose members are strong inducers of epithelial differentiation (and their expression decreased in metastatic cancers) and miR-200 family also inhibited the expression of ZEB1 by binding to miR-binding site of ZEB1 3'UTR. It indicate that miR-200 members and ZEB factors reciprocally control each other in a feedback loop. Considering all this information, we can use the 3'UTR of ZEB1 for specific expression of different therapeutic genes in metastatic breast MDA-MB231 cell line (wich contained more than 90% CSC), because there is no or little expression of miR-200 family that bind to their miR-targets.
Materials and Methods: 3'UTR of ZEB1 gene were amplified and cloned to pmiR-report vector. MDA-MB231 as a metastatic and MCF-7 as a non-metastatic breast cancer cell line have been cultured. Their miRNAs were extracted by high pure miRNA isolation kit and the level of miR-200 family expression quantified by LNA enhanced RT microRNA PCR primer. Both cell lines were seeded in 24-well plates 24 hours prior to transfection. The following day, 200 ng of reporter plasmid along with 200 ng of control Renilla-luciferase plasmid were co-transfected to both cell lines using Lipofectamine 2000. Cells were collected 24 hours after transfection and assayed for luciferase activity using the luminometer.
Results: The results show that the expression of luciferase in MDA-MB231 cell line is much more than MCF-7 cell line.
Conclusion: 3'UTR of ZEB1 could be used for specific expression of therapeutic gene because there is no or very littel expression of miR-200 family so they don't bind to miR-binding site of ZEB1 gene.
Keywords: Epithelial to Mesenchymal Transition, Zinc-finger E-box Binding Homeobox 1, MiR-200 Family

Ps-32: Methylation Patterns of Key Transcription Factors in Osteoblastic Differentiation of Mesenchymal Stem Cells

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Objective: RUNX2 and OSX as two specific and DLX5 as non-specific osteoblast transcription factors are of paramount importance in regulating osteoblast related genes including osteocalcin, BSP, osteopontin and collagen type Iα1. The present research sets out to investigate whether epigenetic regulation of these genes is important in osteoblastic differentiation of MSCs.
Materials and Methods: MSCs were isolated from human BM. They were differentiated to osteoblasts. DNA and RNA were extracted at days 0, 7, 14, and 21 from MSCs differentiating to osteoblasts. Promoter regions of RUNX2, OSX, DLX5 and BSP were analyzed by MSP. Gene expression was analyzed during osteoblastic differentiation by Real Time-PCR.
Results: Promoter methylation status didn't change in RUNX2, DLX5 and BSP during osteoblastic differentiation of MSCs. In contrast, OSX promoter showed a dynamic change of methylation pattern. Moreover, RUNX2, OSX, DLX5 and BSP promoter regions showed three different methylation patterns during MSCs differentiation. Gene expression study confirmed these results.
Conclusion: Epigenetic regulation of some involved genes may play a leading role in differentiation of Mesenchymal stem cells to osteoblast.
Keywords: DNA Methylation, Osteoblastic Differentiation, Mesenchymal Stem Cells

Ps-33: Similar Nucleosome Occupancy Levels of the Histone Variant H2A.Z, in Human Embryonic Stem Cells and Induced Pluripotent Stem Cells

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Objective: Embryonic stem (ES) and induced pluripotent stem (iPS) cells are pluripotent cells indebted their developmental plasticity to their unique structure of chromatin, designated as total epigenome signature. Multiple molecular mechanisms alter chromatin structure and dynamics of cells to facilitate access of regulatory proteins to their genonic DNA. A variant of histone H2A, named H2A.Z, is of epigenetic elements playing important roles in open/closed chromatin formation. Since distribution of H2A.Z is not uniformly throughout the genome and it is usually enriched at promoters of developmentally regulated, incorporation of this variant within chromatin is important for proper gene expression pattern and genome stability of cells through development.
Materials and Methods: In the current study, total levels of histone H2A.Z were quantitatively evaluated using nucleosome-ELISA thechnique, in ESC and iPSC as pluripotent cells, and differentiated-ESC and fibroblast cells as differentiated lines.
Results: Results showed remarkable similarities in H2A.Z-nucleosome occupancy levels of ESC and iPS cells, while this parameter was significantly lower in differentiated-ESC and also fibroblast cells.
Conclusion: Current findings implies a dynamic epigenetic role for H2A.Z in pluripotency and gene regulationmechanisms involved in development and differentiation.
Keywords: H2A.Z, Pluripotency, Epigenome, ES, iPS

Ps-34: Analysis of Over Expression Using Lenti-X-Advanced Inducible System Carrying Mouse Peroxisomal Protein (PEP) CDS to Investigate The Over Expression Effects of PEP during Neural Differentiation of Mouse Embryonic Stem Cells

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Objective: Peroxisomal protein (PEP) is considered to be a peroxisomal matrix protein was previously identified as Fndc5 (firbonectin type III domain containing 5 proein; NCBI accession number: AK137327). As peroxisomes have been reported to have essential functions in brain, this expression pattern suggests a possible role for PEP in process of neurogenesis. Very recently it was reported that PEP could be cleaved and secreted to the outside of cells and acts as a hormone. The secreted portion was named Irisin. Irisin sequences are highly conserved in all mammalian species and are important for thermogenesis. The therapeutic potential of Irisin was raised as it was shown that would be effective in increasing of the diabetic individual tolerance to Insulin. Furthemore, exogenously administered Irisin induced the browning of subcutaneous fat. Our previous report indicated that PEP involved in the early process of neurogenesis. The results of previous study revealed that increased expression of PEP occured during retinoic acid induction when embryoid bodies were formed. In this study, we have designed a vector for efficient overexpression of PEP in process of neural differentiation whether it affects this process.
Materials and Methods: An appropriate lentiviral vector, pLVX-Tight-Puro, containing the coding sequence of PEP was constructed as reported previously. Then mouse embryonic stem cells were transduced with lentiviruses derived from the pLVX-Tet-Off Advanced regulator vector. Transgenic mouse embryonic stem cells were selected with G418 for 14 days. To check the efficiency of lentiviral vectors, transgenic mouse embryonic stem cells which expressed tTA were transduced further with pLVX-Tight-Pur-EGFP vector. The stable line was selected by both of G418 and puromycin application. Overexpression of EGFP was analysed for with flowcytometry and real time PCR. After ensuring of the viral vector function, stably tTA expressing mouse embryonic stem cells were transduced with pLVX-Tight-Pur-PEP vector. Stable cell line selection carried out with G418 and purimycin.
Results: The lentiviral vector expressing PEP was constructed correctly as checked by sequencing. Moreover results indicated stably expression of PEP in mouse embryonic cell line which was induced after doxycyclin treatment.
Conclusion: Thus we have generated a stable mESCs overproducing PEP which is ready for further analyses.
Keywords: Mouse Embryonic Stem Cell, Inducible Lentivirus Vector, Neural Differentiation

Ps-35: Influences of PPARγ and PEP on Proliferation and Differentiation of Mouse Embryonic Stem Cells

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Objective: Peroxisome proliferator-activated receptor γ (PPARγ) belongs to the nuclear receptor-dependent ligand-activated transcription factors that regulate ex-

pression of many genes involved in metabolism, cell differentiation, adipogenesis, inflammation, and apoptosis. In this study, we have examined the influences of PPARγ in presence or absence of LIF, on mESCs proliferation. Moreover, the importance of PPARg in neural differentiation of mouse embryonic stem cells (mESCs) was assessed. Furthermore, peroxisomal protein (PEP), a peroxisomal matrix protein comprising 209 amino acid residues, was initially cloned by Ferrer-Martinez in 2002 and by our group in 2009. The expression of peroxisomal protein (PEP) during neurogenesis was also examined. On the other hand, the PEP knockdown was carried out in order to see the importance of its expression during the neural differentiation. Finally the PPARγ and PEP promoters were cloned and their nucleotide elements were analyzed.

Materials and Methods: Mouse embryonic stem cells (mESCs) were implemented in whole of our studies as an *in vitro* model cells. Influences of PPARγ on cell proliferation were examined by its activation and inactivation through using its agonists (Rosiglitazone and Ciglitazone) and antagonist (GW9662) in two states of LIF presence and absence. Additionally JAK/STAT3 and ERK signaling pathways were tracked under these conditions. To testify the importance of PPARγ in neural differentiation, at first step, the expression level of PPARγ was assessed during different steps of neural differentiation. Meanwhile, involvement of PPARγ in stages of neural differentiation was examined by application of its agonist and antagonist. To study the expression of PEP during neurogenesis, expression pattern of PEP was investigated under distinct steps of neural differentiation by means of RT-PCR and real-time PCR. As a further look to the function of PEP which might be involved in neurogenesis. In the present study, we constructed an inducible short hairpin RNA (shRNA) vector that is expressed under induction by doxycycline (Dox). Next, we generated a stably transformed mESCs line that expressed shRNA against PEP. The PEP knockdown was carried out in two stages of neural differentiation of mESCs; during and post-neural progenitor (NPs) formation. In this project, we have identified PEP and PPARγ promoters using cloning of the putative core promoter sequences upstream of the EGFP reporter gene in eukaryotic expression vector and analyzed them besides of their elements upon transfection in to several mammalian cell lines using flow-cytometry and fleurocent microscopic analyses.

Results: We demonstrated that in the presence of LIF, the activation of PPARγ by its agonists (Rosiglitazone and Ciglitazone) led to increased mESCs proliferation whereas PPARγ antagonist (GW9662) reversed this effect. Additionally, upon PPARγ activation, LIF increased PPARγ expression and resulted in degradation of suppressor of cytokine signaling 3 (SOCS3), an important negative regulator of the LIF/ signal transducers and activators of transcription 3 (STAT3)-pathway. In the absence of LIF, PPARγ agonists decreased proliferation of mESCs. In this state, extracellular signal-regulated kinase (ERK) proteins that were activated through phosphorylation resulted in the suppression of Nanog expression, an important pluripotency determinant, but did not affect Oct4 expression at both the RNA and protein levels. However, treatment of undifferentiated mESCs with PPARγ agonists had no influence on further differentiation. On the other hand, data revealed an elevation in expression level of PPARγ when neural precursors (NPs) are formed upon retinoic acid treatment. Our results indicated that PPARγ inactivation via treatment with GW9662 during NPs formation, reduced expression of neural precursor and neural (neuronal and astrocytes) markers. However, PPARγ inactivation by antagonist treatment post-NPs formation stage only decreased the expression of mature astrocyte marker (Gfap) suggesting that inactivation of PPARγ by antagonist decreased astrocyte differentiation. Furthermore, expression pattern of PEP transcripts were markedly increased after the RA treatment at embryoid body and neural stages. Also our results indicated that in the process of NPs formation, the decrease in PEP expression significantly reduced expression of NPs and mature neuronal markers which modulated neuronal differentiation. A decrease in PEP expression during the post-NPs formation stage also caused significant reduction in the levels of mature neuronal markers. In this project, we cloned PEP promoter and transferred it into CHO, P19 and mESCs which showed to be active. We elucidated this promoter activity during neurogenesis upon RA and Noggin treatment using of the stable mESCs transfected with PEP promoter-EGFP containing vector and determined functionality of Vitamin D response elements (VDRE) in PEP promoter which was predicted by bioinformatics analysis. The promoter of PEP gene is TATA-less type and lacks a consensus initiator sequence. Vitamin D treatment caused down-regulation of both EGFP and endogenous PEP expression. The promoter region of PPARγ gene was also cloned. Data indicated that in promoter of PPARγ1 retinoid X receptor and Vitamin D receptor response elements are present which could modulate PPARγ gene expression pattern during differentiation processes.

Conclusion: Decreased cell proliferation in the presence of PPARγ agonist may be attributed to a decrease in Nanog expression in the absence of LIF, which resulted in cell differentiation. Thus, the PPARγ antagonist reversed the effects of the agonists in both the presence and absence of LIF. We can conclude that PPARγ may have a dual role in the presence or absence of LIF on the proliferation and differentiation states of mESCs. On the other hand, we have demonstrated the stage dependent role of PPARγ modulation on neural differentiation of mESCs by retinoic acid treatment. As our results indicated an increased expression of PEP transcripts after RA treatment during neural differentiation, we concluded that PEP might be involved in the early process of neurogenesis. Moreover, the conditional expression of PEP RNAi modulated neural differentiation rather than cell viability and proliferation.

This modulation was seen for neuronal and astrocytes generation as the related markers expressions were efficiently suppressed under this condition. We concluded that PEP expression is required for the appropriate neural differentiation of mESCs.
Keywords: PPARγ, Peroxisomal Protein, Neural Differentiation, Promoter, mESCs

Ps-36: Effects of Zinc Ion on Creation of Male Germ Cells Properties in Sheep Marrow-Derived Mesenchymal Stem Cells

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Objective: From the distant past, many studies have shown the effects of zinc on male infertility. Zinc is a well-known essential trace element that plays a unique role in polymeric organization of macromolecules like DNA and RNA, DNA transcription, expression of steroid receptors, proteins synthesis, and cell division. Zinc is involved in the activation and maintenance of the germinal epithelium of semineferous tubules and able to affect germ cells for more deviation and differentiation. This is the first report about the effects of zinc ion on expression of male germ cells features in mesenchymal stem cells.
Materials and Methods: Passage-3 male sheep marrow-derived mesenchymal stem cells treated with 0.14 µg/ml zinc sulfate (ZnSO4) for 21 days. Expression of male germ cells features was evaluated by assessment of germ cells common features include expression of germ cell-specific markers like VASA, PIWIL2, OCT4, beta1 INTEGRIN, DAZL (by RT-PCR, qRT-PCR) and PGP 9.5 (by immunocytichemistry), morphological characteristics and changes of the cells alkaline phosphatase activity.
Results: No morphological changes were seen by treatment. Although VASA, PIWIL2, OCT4 and beta1 INTEGRIN were expressed in control group, but the expression of VASA and beta1 INTEGRIN were upregulated and PIWIL2 and OCT4 were downregulated. DAZL and PGP 9.5 weren't expressed and alkaline phosphatase activity did not change after zinc treatment.
Conclusion: These results show that although ZnSO4 doesn't induce germ cell differentiation in mesenchymal stem cells but upregulation of VASA and beta1 INTEGRIN and OCT4 downregulation, suggests that maybe one of the mechanisms by which zinc ion can increase male fertility is help to regulate the expression of some specific genes in male germ cells of the testis and their differentiation process.
Keywords: Zinc Ion, Male Fertility, Mesenchymal Stem Cells, Germ Cells

Ps-37: Sonic Hedgehog Inhibition Affects Fate of Mesendoderm Differentiation of Mouse Embryonic Stem Cells

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Objective: Sonic Hedghoge (Shh) affects both development of embryo and also embryonic stem cells (ESCs). This research was aimed to identify the effect of Shh inhibition during definitive endoderm differentiation of mouse embryonic stem cells.
Materials and Methods: Induced embryoid bodies (EBs) from mESC line MUKF3 treated with Hedgehog interacting protein (HIP; 10 µg/ml) for 5 days in experimental group. Then, EBs was cultured on gelatin-coated dishes in the attached position for an additional 8 days. Finally, total RNA of control and experimental groups were extracted and the expressions of undifferentiated, ectoderm, mesoderm and endoderm markers were compared by relative RT-PCR and statistically analysed. The definitive endoderm cells identified by immunohistochemistry staining of goosecoid (GSC) of the treated EBs.
Results: There was a significant decrease in expression of brachury, goosecoid, GATA4 in experimental groups ($p<0.005$). Expression of nestin, PDX-1, TAT in the experimental groups were significantly increased ($p<0.005$). Results of immunohistochemistry staining revealed an decrease in the ratio of GSC positive cells in the treated groups.
Conclusion: Shh inhibition leads to further differentiation of mesendodermal lineages towards definitive endoderm besides mesoderm in mESC.
Keywords: Definitive Endoderm, Hedgehog Interacting Protein, Mesendoderm, Mouse Embryonic Stem Cell Definitive Endoderm, Hedgehog Interacting Protein, Mesendoderm, Mouse Embryonic Stem Cell

Ps-38: Evaluation of Collagen Membranes with Autologous Keratinocytes on Open Wound Healing in the Horse

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Objective: To use of autologous equine keratinocyte with keratinocyte collagen membrane grafts method (KCMG) for treatment of wounds of the distal aspect of the horse limb.
Materials and Methods:
Study Design: Full thickness excision wounds (2.5×2.5cm) were created on the lateromedial of both metatars of each horse aseptically. The wounds were classified into two groups, group A and B as control (n=4) and keratinocyte collagen membrane graft (KCMG [n=4]) group respectively. After four days, cell graft was preformed. Whole wounds excision biopsies were performed on 28th day after wounding for histopathological evaluation.
Animals: Four healthy adult horses (Mean: 368 kg).
Method: Keratinocyte cells have been separated by enzyme digestion from lib skin samples. Acid soluble collagen has been extracted from calf skin and polymerized *in vitro*, then keratinocyte cells that have been proliferated *in vitro*, cultured on collagen membrane for one day. Treatment was done once on the 4th day.
Results: Geometrically, there was increase in epithelialization, contraction and total wound healing per day in group B, but it is not significant. Histopathologically both groups had epidermal cells in superficial layer, but the amount of this layer, differentiation and maturation of stratum spinosum cells of epiderm in group B was better than group A.
Conclusion: For increase of cell treatment effects significantly, it is better to use cell grafts on large wound, also preparation of wound bed and repeated treatments are essential.
Clinical Relevance: Use of keratinocyte collagen membrane graft (KCMG) have positive effects and cause better wound healing in derm and specially epiderm of equine lower limb wounds.
Keywords: Keratinocyte, Collagen Membrane, Horse, Limb, Wound Healin

Ps-39: The Evaluation of HHV-8 among Cord Blood Donors by Real Time PCR

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Objective: Cord blood is one of the most important sources of hematopoietic stem cells which can be used for transplantation to cure malignant and non-malignant diseases because of lower risk of GVHD. Transplanting CB stem cells might cause infections in recipients. HHV8 is a γ-herpes virus that infects circulating mononuclear cells in blood. This virus is the main agent of Kaposi`s sarcoma and can reject transplantation. It is known that, HHV8 can transfer during sex among homosexual men, saliva in children and blood products. The aim of this study is to verify vertical transmission of HHV8 during pregnancy by cord blood in order to improve usage of cord blood stem cells in all medical aspects.
Materials and Methods: To assess the incidence of HHV8 infection by CB, we surveyed 800 cord blood samples of women by the mean age of 26. After extracting DNA of CBMCs (Cord Blood Mononuclear Cells), Real Time PCR was done in 40 cycles for all samples. At the end RT-PCR and Real Time PCR was performed for HHV8 positive CB samples.
Results: The overall HHV8 seroprevalence in cord blood mononuclear cells was 1.38% . Only 11 pregnant women represented DNA of HHV8 in their cord blood. Results of Real Time PCR for cDNAs of positive samples demonstrated no lytic phase of HHV8 in our CB samples.
Conclusion: Our data illustrate that screening suspicious CB samples and studying on more samples may eliminate risk of HHV8 transmission through CB transplantation.
Keywords: HHV8, Cord Blood, Stem Cell

Ps-40: Isolation and Culture of Mesenchymal Stem Cells from Wharton Jelly of Human Umbilical Cord

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Objective: The Wharton jelly of human umbilical cord contain multipotent stem Cells that have basic properties of mesenchymal stem cells (MSCs) such as the capacities to self-renew indefinitely and differentiate into multiple cells and tissue types.
Materials and Methods: Human umbilical cord for this study was aseptically collected from full-term infants delivered by caesarian section that carried in the sterile salt solution0.9% to laboratory. Then removal vessels of umbilical cord divided to pieces smaller about 1 mm and were cultured in DMEM. The passage-4 cells were analyzed with CD49, CD13, CD90 markers that indicated these cells had a mesenchymal stem cell (MSC) identity. We assessed the cells for Alkaline Phosphatase activity to investigate the mesenchymal (stromal) nature. Finally, the cells were differentiated into the adipocytic lineages in different subcultures and analysed by Histochemical staining.
Results: MSCs in the Wharton's Jelly were easily attainable and could be maintained and expanded in culture.Flow cytometry resulte showed that the cells expressed high level of mesenchymal stem cell mark-

ers. The alkaline phosphatase activity of Humscs were positive. Adipogenic differentiation demonstrated by as seen Oil red O–positive cell.
Conclusion: MSCs can be easily obtained without causing pain to donors, and the procedure avoids ethical and technical issues from wharton's jelly of human umbilica cord.
Keywords: Mesenchymal Stem Cell, Whartan's Jelly, Flow Cytometry, Differentiation Cell

Ps-41: Role of Hypergravity and Microgravity in The Differentiation of Bone Marrow Stromal Cells

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Objective: Bone marrow stromal cells (BMSCs or MSCs) are adult stem cells that have the potential for transdifferentiation into different cell types and carry the potential for repairing degenerative injuries and diseases. For functional recovery by these cells, the grafted cells must secrete the required complement of factors for repair of the damaged region. BMSCs normally give rise to bone, cartilage, and mesenchymal cells. In recent years, bone marrow cells have been shown to have the capacity to differentiate into myocytes, hepatocytes, adipocytes, beta-pancreatic islets cells, glial cells and neural cells under specific experimental cell culture conditions by using appropriate growth factors. Recently it has shown that different condition of mechanical force and gravity can regulate differentiation of BMSCs through various signaling pathways, too.
Materials and Methods: For hypergravity experiment, BMSCs were cultured on a cell centrifuger to obtain hypergravity and for simulating microgravity; cells were cultured on a clinostat. Then both samples used to differentiate into cardiomyocytes, osteoblasts and adipocytes.
Results: Studies have shown that hypergravity promotes BMSC differentiate into force-sensitive cells, cardiomyocytes and osteoblasts, whereas microgravity promotes differentiation into force-insensitive cells, namely adipocytes. Also hypergravity promoted BMSCs proliferation and microgravity inhibited proliferation of them. Studies have shown that gravity condition affects mRNA expression, too. In BMSCs, hypergravity increase the expression of cardiomyocytes and osteoblasts markers but decrease the expression of the adipocytes markers.
Conclusion: All together gravity is an important factor affecting the differentiation of BMSCs. This provides a new window for studies of stem cell differentiation and new approach in cell therapy and regenerative medicine.
Keywords: BMSC, Hypergravity, Microgravity, Differentiation

Ps-42: Attenuation of Neural Differentiation Follow Up PEP Gene Knock Down in Mouse Embryonic Stem Cells

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Objective: Peroxisomal protein (PEP) is one of the peroxisomal matrix protein comprises 209 aminoacid residues. Our recent data indicated that PEP expression level was increased upon retinoic acid treatment during neural differentiation of mouse embryonic stem cells. In order to further look in to the PEP function during neural differentiation, a gain off function of PEP performed by means of RNAi approach to knock down its expression.
Materials and Methods: Several siRNAs was designed using different bioinformatics softwares to silence PEP gene expression. The most appropriated siRNAs with length of 19 nucleotides, were selected along with a negative control siRNA(scramble). oligonucleotides were inserted into pSingle tTS-shRNA inducible vector at HindIII and XhoI sites. Recombinant vectors were transfected into the mouse embryonic stem cells. Stably transformed colony cells expressing PEP shRNA were selected after antibiotics treatment. Doxycycline was used to induce the production of PEP shRNA. Cells were allowed to differentiate. Real Time PCR used for analysis gene expression based on $\Delta\Delta$ ct methods.
Results: Real time quatitative RT-PCR and western blot revealed that PEP knock down significantly decreased the expression level of neuron markers(Map2,NeuN) and astrocyte marker (Gfap) during neural differentiation.
Conclusion: These data confirmed a possible role of PEP during neural differentiation.
Keywords: PEP, RNAi, shRNA, Mouse Embryonic Stem Cell

Ps-43: Immunomodulatory Effects of Mouse Adipose-derived Mesenchymal Stem Cells is Strain-dependent

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Objective: Mesenchymal stem cells (MSCs) are mesoderm-derived stromal cells that are capable of differentiating *in vitro* and *in vivo* to mesenchymal lineages. Several recent reports have demonstrated that MSCs have immunomodulatory or immunosuppressive effects on many immune cells. Since the selection of ideal stem cell is apparently crucial for the outcome of experimental stem cell therapies.
Materials and Methods: In this study we compared cytokines, NO, and IDO production and immunomodulatory effects of adipose-derived MSCs (AD-MSCs) isolated from BALB/c, C57BL/6 and DBA mouse strains.
Results: No significant difference was found in the morphology, cell surface markers, *in vitro* differentiation and proliferation potentials of AD-MSCs isolated from C57BL/6, BALB/c and DBA mice. The immunological assays showed some variation among the strains in the cytokines, NO, and IDO production and immunomodulatory effects on splenocytes functions. Our results indicated that BALB/c conditioned media exerted a higher suppression of splenocytes proliferation. AD-MSCs isolated from C57BL/6 and BALB/c mice produced higher levels of TGF-β than those from DBA mice. Furthermore, IL-17 and IDO production was higher in AD-MSCs isolated from BALB/c mice. Our results indicated an increased production of TGF-β, IL-4, IL-10, NO and IDO production by splenocytes in response to conditioned media from BALB/c AD-MSCs.
Conclusion: Our results showed that the immunomodulatory properties of mouse adipose tissue-derived mesenchymal stem cells is strain-dependent.
Keywords: Immunomodulatory Effects, Mouse, Adipose-derived Mesenchymal Stem

Ps-44: The Promotion of Stemness and Pluripotency of Mouse Embryonic Stem Cells Following Feeder-Free Culture on Collagen-Grafted Polyethersulfone (PES) Nanofibrous Scaffold

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Objective: Derivation, proliferation, and pluripotency of ESCs depend on their microenvironment, including the chemical and physical properties of the extracellular matrix (ECM) and the presence of growth factors. Surface modification of electrospun nanofibrous scaffolds have been studied to closely resemble natural ECMs and support *in vitro* and *in vivo* proliferation, pluripotency and differentiation of stem cells.
Materials and Methods: In this study, we analyzed the maintenance of stemness and pluripotency of the mouse embryonic stem cell (mESC) following feeder-free culture on collagen-grafted polyethersulfone (PES-COL) electrospun nanofibrous scaffold.
Results: Our results showed that, the mESCs cultured for seven passages on PES-COL scaffolds had a typical undifferentiated morphology, enhanced proliferation, stable diploid normal karyotype, and continued expression of stemness and pluripotencyassociated markers, Oct-4, Nanog, SSEA-1, and Alkaline phosphatase (ALP) in comparison with PES scaffolds and gelatin-coated plate. Moreover, these cells retained their *in vitro* and *in vivo* pluripotency. Our results indicated the enhanced infiltration and teratoma formation of mESCs in PES-COL.
Conclusion: Here we demonstrate a successful feeder-free culture system in which mESCs can be maintained an undifferentiated state and thereby eliminating the need of feeder layers for *in vitro* culture of embryonic stem cells. Furthermore, collagen-grafted nanofibrous scaffold could be a good candidate for feeder-free culture of human embryonic stem cells and induced pluripotent stem cells for clinical application.
Keywords: Embryonic Stem Cell, Feeder-free, Pluripotency, Polyethersulfone, Nanofiber, Surface Treatment

Ps-45: The Effect of Mitomycin-C on Transfection Ratio in Unrestricted Somatic Stem Cells-Derived Umbilical Cord Blood

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Objective: Nowadays, transfection in Mesenchymal Stem Cells is an alternative way to achieve differentiated cells for clinical approach. Unrestricted somatic stem cells (USSCs) as mesenchymal progenitor cells (MPCs), have widely potential for this goal. In some cases they were use as a feeder layer and for inhibition of cross transfection, specific vector were used. We want to show that simply treatment like a Mitomycin-C could decrease cross transfection.
Materials and Methods: USSCs were isolated from cord blood units and characterized by morphological and immunological analysis for CD166, CD73, CD105, CD90, CD44, CD117, CD34, CD45, KDR and Cadherin V. In order to confirm the source USSCs as human MPCs, the differencing potential of USSC into adipocytes and osteoblasts by specific factors and medium were check. Then cells in 2 group: control and treated

by Mitomycin-C were transfected by scramble siRNA and BLOCK-iT™ Alexa Fluor Red Fluorescent Oligo. After 72 hours Quantitative real-time PCR were done.
Results: Population Doubling Time (PDT) of USSCs wasn't changed during the experiment periods of primary culture to passage 30 (49.48 ± 0.3 hours). A fluorescent microscope showed 99% decrease in transfection ratio in treated group but qRT-PCR indicated less than 8.7% ± 0.1% ($p<0.05$) effect on gene expression. Finally flow-cytometry analysis showed no significant difference in the percentage of CD marker.
Conclusion: Mitomycin-C were inhibited the mitotic activity of cells. In this way, cellular pores were blocked for no signaling, so that their transfection ratio was decreased. For mimicry of *in vivo*, target cells were *ex vivo* co-cultured on the feeder layer. These target cells can be transfected by RNAi delivery system and we would be worry about RNAi going into the feeder layer cells, too (cross transfection). But when the feeder layer was treated by Mitomycin-C, RNAi almost couldn't enter them.
Keywords: Mitomycin-C, Unrestricted Somatic Stem Cells, Cord Blood

Ps-46: Isolation, Culture and Transplantation of Neural Progenitor Cells from Rat Cerebellum at Postnatal Day1

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Objective: Neural stem cells have been identified in specific areas of the mature brain as well as in the cerebellum. In order to characterize and determine the therapeutic potential of adult neural progenitor cells (NPCs), it is important to be able to isolate and study NPCs from the cerebellum, in which there are suitable for transplantation in cerebellar degeneration models. The focus of this study was to characterize the cultivation, differentiation and transplantation of adult rat NPCs isolated from the cerebellum.
Materials and Methods: NPCs of the cerebellum of postnatal day 1 (PND1) rats were isolated by enzymatic digestion and cultured for generation of primary neurospheres under the appropriate conditions. Spheres were dissociated and examined for proliferation capacity using CFU-F assay. Then, NPCs analyzed for capacity for differentiation and surviving after transplantation.
Results: NPCs form spherical clusters are mechanically dissociated to a single-cell expressing Nestin, Olig2, NFM and SOX2, but do not express Musashl1 in passage 4. Differentiated neural progenitor cells express Nestin and A2B5 in flowcytometery analysis. Cultured adult rat NPCs also survived 10 weeks after transplantation into the cerebellum of the normal adult rat brain.
Conclusion: We have optimized approach that allow for the routine isolation, culture, and transplantation of NPCs derived from the cerebellum at PND1.
Keywords: Cerebellum, NPCs, Isolation, PND1, Transplantation

Ps-47: Effects of Lipopolysaccharides from Different Shigella Strains on Toll-Like Receptor 4 Expression in Human Bone Marrow Mesenchymal Stem Cells

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Objective: Toll-like receptors (TLRs) are a conserved family of receptors that recognize pathogen-associated molecular patterns and promote the activation of immune cells. To date, several TLRs (numbered 1–11) have been identified in humans. One such receptor–ligand complex is formed between the mammalian Toll-like receptor 4 (TLR4)–MD2–CD14 complex and bacterial lipopolysaccharide (LPS). Initially, research on TLRs focused on their expression and signaling consequences in immune cells. However, recent reports indicate that other bone marrow-derived cells, including mesenchymal stem/progenitor cells (MSCs), are among the cells that express TLR proteins. MSCs are separated from other cells in the bone marrow by their tendency to adhere to plastic.
Materials and Methods: In this study, the effects of LPS from different shigella strains (S. flexnery, S. dysentriae and S. sonnei) with various concentration include of 0.1, 1 and 10 µg/µl on TLR4 expression in human bone marrow derived mesenchymal stem cell was evaluated by RT-PCR and Q-PCR.
Results: RT-PCR has not shown TLR4 expression in control group in contrast of all case group. Q-PCR data has shown all of lipopolysaccharides from shigella strains with varies concentration led to TLR4 expression. However, the highest expression was shown in 10µg/µl of S.flexneri treatment and the lowest one in 0.1 µg/µl shigella sonnei treatment.
Conclusion: The innate immune response induced by Gram negative bacterial LPS as a TLR4 agonist. LPS

composition varies widely among different species of Gram negative bacteria. Therefore, it expected that LPS from different shigella strains is leading to different expression levels of TLR4. Our data confirm this expectation. In addition, Q-PCR data was shown ascending levels of TLR4 expression with increasing of LPS concentration from all of shigella strains.

Keywords: Human Bone Marrow Derived Mesenchymal Stem Cells, TLR4, LPS, Shigella

Ps-48: Effects of Adipose-Derived Mesenchymal Stem Cells Pre-Ischemic and Post-Ischemic Injection on Brain Stroke in Rats

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Objective: Mesenchymal stem cells are multipotent cells that can be isolated from different tissues such as bone marrow and fat tissue. Some cytokines such as TNFα and IL-8 released in brain infarction induce strong inflammation and infarction. On the other hand cytokines and growth factors secreted from Mesenchymal stem cells (MSCs) could protect cerebral ischemia. The goal of this study was to compare the effect of injection of MSCs on infarction volume before and after inducing brain ischemia in rats.

Materials and Methods: Adipose-derived mesenchymal stem cells were isolated from 2×3 cm fat tissue enzymetically dissociated by collagenaseI 0.15% which their multi-potentency was confirmed by differentiating to osteocyte and adipocyte. Focal cerebral ischemia model was performed with Koizumi's method in 15 rats (5 rats for control group, 5 rats for pre-ischemic injection and the rest for post-ischemic injection group). Briefly External carotid artery (ECA), Common carotid artery and internal carotid artery were exposed. ECA was clamped and a 4.0 monofilaments nylon suture with rounded tip was passed through ICA until it blocked the root of middle cerebral artery, after 45 min the monofilament was removed and blood reperfused. In control group (first) only 200λPBS was injected, in pre-ischemic group(second) 200λ PBS containing cells/ml was injected 1 day before inducing ischemia and in post-ischemia (third) group 200λ PBS containing cells/ml was injected 1 day after inducing ischemia. All injections were intravenous through tail vein. After 28 days brain was removed and stained with TTC (2, 3, 5-triphenyltetrazolium chloride) to define infarction volume.

Results: Rats' brains in third group had the least infarct volume and rats' brains in second group had lesser infarct volume than the first one.

Conclusion: Transfusion of Mesenchymal stem cells after brain ischemia had the best effect because of their anti-inflammatory and supportive effects.

Keywords: Mesenchymal Stem Cell, Brain Ischemia, Anti Inflammatory

Ps-49: Comparative Analysis of Growth Characterization of Stem Cells Derived from Human Dental Pulp Polyp (PPSCs) versus Normal Dental Pulp Stem Cells (DPSCs)

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Objective: Dental pulp stem cells (DPSCs) have been shown to have more replicative potentials in comparison to bone marrow mesenchymal stem cells (MSCs) while having the same differentiation capacity. We have previously investigated the isolation of stem cells from pulp polyps (chronic hyperplastic pulpitis), a pathological tissue produced in an inflammatory proliferative response within a tooth supplied by a very good blood flow. The Goal of this study was to assess growth characterization analysis of these cells and compare them to DPSCs.

Materials and Methods: Pulp polyps of eight permanent molar teeth achieved through simple curettage of pulp chamber. Four samples of healthy wisdom dental pulps were taken as the control. The tissues were digested by collagenase and dispase. Single cells were harvested and cultured. To compare the growth characteristics of polyp derived stem cells and DPSCs, 3rd passage cells from healthy pulps and polyps were seeded with the concentration of 2×10^4 cells/well in all the wells of 12-well culture plates. Every day cells from one of the wells were detached by Trypsin-EDTA and counted with hemocytometer. Based on the results we draw growth curve and population doubling time was calculated by the formula "logN2-logN1/ log2" in which the N1 shows the number of cells in the beginning of the log phase of growth and N2 shows the number of cells at the end of the log phase.

Results: Cells isolated from pulp polyps displayed similar features regarding morphological characteristics DPSCs do. The pulp polyp derived cells grew faster than DPSCs. The mean for population doubling time for pulp polyp derived cells is 1.13 days and for DPSCs is 1.57 days (p = 0.086).
Conclusion: Pulp polyps are easily accessible non-invasively acquired tissue resources which contain adequate number of cells with a faster growth pattern in comparison to DPSCs.
Keywords: Adult Stem Cells, Dental Pulp Stem Cell, Pulp Polyp, Chronic Hyperplastic Pulpitis, Pulp Polyp Stem Cell, Population Doubling Time Analysis

Ps-50: Derivation of Definitive Endoderm from hiPSCs Cultured on PLA Nanofibrous Scaffolds

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Objective: Over the last two decades, whether cell replacement therapy can overcome the using of insulin administration in diabetes mellitus type 1 (IDDM) patients or not, has became a challenging issue amongst clinicians and cell biologists. The idea to generate human induced pluripotent stem cells (hiPSCs) specific from diabetic patient and differentiated to insulin-producing cells pioneered by Melton's lab, has opened a new window to using of iPS cells as new source for cell therapy of diabetes without warring about the immunological rejection or ethical issues. Use of 3 dimensional (3D) scaffolds is one access to potentially enhance common differentiation protocols, which has been shown to improve cellular function and differentiation potential.
Materials and Methods: In the present study we intended to using pluripotent hiPS seeded onto Matrigel coated 3D poly lactic acid (PLA) scaffolds to derive definitive endoderm cells for 7 days, the first crucial stage of endoderm-derived tissues differentiation by use of Activin A with concentration of 100 ng/ml and Wnt3a with concentration of 25 ng/ml. We used immunofluorescent and RT-PCR methods for survey experiments.
Results: Our results showed that hiPS which were coaxed to differentiate on Matrigel coated 3D scaffolds can be in prosperous manner enticed to differentiate into definitive endoderm. The immunofluorescent study and RT-PCR results revealed that iPS cells that were cultured on Matrigel coated 3D scaffolds expressed significantly higher levels of the key endoderm transcription factors SOX17 and GSC in comparison to those differentiated on 2D cultures.
Conclusion: Our research confirmed the positive effect of 3D cultures on endoderm commitment of hiPS. The result of this study may have impact in future therapy of IDDM patien by cell replacement therapy.
Keywords: hiPS, Differentiation, Poly Lactic Acid, Scaffold, Definitive Endoderm, Pancreas

Ps-51: Investigation of Neural Growth Factor Gene Expression in Unrestricted Somatic Stem Cells

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Objective: Delivery of neurotrophic factors to the site of injury may promote neural cells repair after peripheral or central injury, therefore the transfer of the stem cells producing this kind of factors could be an effective method with respect to injury repair. Unrestricted somatic stem cells (USSC) are non-hematopoietic stem cells isolated from the umbilical cord blood and are appropriate using in cell therapy. Different studies, *in vitro* and vivo models have shown the ability of these cells to support or trigger the neuronal/Axon regeneration. To identify the molecular mechanisms of this effect, the expression of some neurotrophic factors such as BDNF and NT-3 have been investigated by different groups. Addressing the importance of the neural growth factor (NGF) in neuronal and axon regeneration, we investigated the expression of NGF in USSCs in this study.
Materials and Methods: Unrestricted somatic stem cells isolated from Umbilical cord blood, characterized by different markers, and cultured in DMEM with 10% FBS and 1% Penicillin- Streptomycin. Real time PCR and ELISA were used to determine the NGF expression in USSCs and Co-culture was done to verify initial results.
Results: Although real time PCR results showed the USSCs express NGF and some other neurotrophic factor genes such as BDNF and GDNF, ELISA results revealed that USSCs didn't express NGF protein. Cell culture medium co-culture with PC-12 model cells showed that USSCs culture medium didn't have any effect on PC-12 cells, as a NGF dependant cell line, and couldn't induce differentiation in these cells.
Conclusion: We conclude that USSCs don't release functional NGF protein; therefore, USSC's effects on neuronal regeneration induction would be related to the expression of other trophic factor proteins such as BDNF, GDNF or NT-3.
Keywords: NGF, Expression, USSC

Ps-52: Comparative Assessment of Toll-Like Re-

ceptor-4 Expression in *In Vitro* Differentiated and *In Vivo* - Derived Odontoblasts

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Objective: Human dental pulp stem cells (hDPSCs) as the multipotent mesnchymal stromal cells (MSCs) possess extensive properties such as accessibly, high proliferation and also muli-lineages differentiation capacity. In addition, these cells are known as the immunoprivileged as well as immunosuppressive for allogeneic transplantation. They are some controversial studies which are indicated the immunogenicity of differentiated allogenous MSCs *in vivo* and/or *in vitro*. Recently, it has been suggested that innate immune receptors called Toll-like receptors (TLRs), as the immune sentinels, contribute to the immunogenicity of MSCs. The aim of this study is to investigate the expression of TLR4 in *in vitro* differentiated odontoblasts in comparison with *in vivo*-derived odontoblasts.
Materials and Methods: hDPSCs were enzymatically isolated from wisdom teeth (n=5) and characterized in terms of surface epitopes (MSC markers CD90/CD73/CD44/CD105 and hematopoietic/endothelial markers CD34/CD45/CD11b/CD31) as well as differentiation capacity into bone, cartilage and adipose cells. Then, hDPSCs were differentiated into odontoblast for 21 days in differentiation medium. Q-PCR was used for comparative TLR4 gene expression in *in vitro* differentiated odontoblasts with the *in vivo*-derived odontoblasts.
Results: hDPSCs showed MSC phenotype and differentiation capacity into three mesenchyme lineages. Immuno-phenotyping results confirmed the existence of MSC markers and the lack of hematopoietic/endothelial markers. Interestingly, Q-PCR results showed significant increasing expression of TLR4 gene in *in vivo*-derived odontoblasts in comparison with *in vitro* differentiated ones.
Conclusion: According to some existing studies about the immunogenicity of *in vitro* differentiated cells, significant less expression of TLR4 in *in vitro* differentiated odontoblasts in comparison with *in vivo* odontoblasts may shed light to the involvement of TLR4 in immunogenicity of MSCs in tissue homeostasis, Especially for the purpose of transplantation.
Keywords: Multipotent Mesenchymal Stromal Cells, Immunogenicity, Innate Immunity, Toll-Like Receptor-4

Ps-53: Isolation and Culture of Human Synovium-Derived Mesenchymal Stem Cells

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Objective: It is possible for synovium tissue to have stem cells. The synovium tissue has a high regenerative capacity, as evidenced by its full healing after surgical and chemical synovectomy in rabbits. With regard to the availability of synovium derived MSCs for clinical use, synovium can be obtained arthroscopically with a low degree of invasiveness and without causing complications at the donor site due, to its high regenerative capacity. The small sample of synovium harvested with a punch biopsy would be sufficient to obtain synovium-derived MSCs for future treatments. The aim of this study was isolation and culture of human synovium-derived mesenchymal stem cells.
Materials and Methods: Samples were provided from synovium tissue of acl (anterior cruciate ligament) and baker cyst paitents. After collagenase digestion cells passed through 70 μm nylon filters and were plated at an appropriate density for expansion. After the 4th passage SM-derived cells were treated in osteogenic and adipogenic medium. Then alizarin red,oil red-o staining and RT-PCR was done.
Results: The isolated cells showed fibroblast-like morphology and have a high proliferation capacity. Calcium deposits, detected by alizarin red staining and Adipogenic differentiation was demonstrated by the accumulation of lipid vacuoles . RT-PCR analysis showed the expression of osteogenic and adipogenic genes.
Conclusion: Our study demonstrates that human multipotent MSCs can be isolated from the Synovial Membrane of knee joints without pain and infection. These cells have the ability to proliferate extensively in culture, from the standpoint of utility as well as high differentiation ability, the synovium is thus an excellent source of MSCs and can be a candidate for developing novel cell based therapeutic approaches for postnatal skeletal tissue repair.
Keywords: Isolation, Human, Stem Cell, Synovium

Ps-54: Laminin Coating Stimulates Cytochrome P450 Enzyme Activity in Hepatocyte-Like Cells Differentiated from Human BM-MSCs

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Objective: CYP450 is considered as one of the most vital metabolizing enzymes in hepatic detoxification, se-

creted by the functional liver cells, termed hepatocytes. Several aspects of hepatocyte-like cells derived from mesenchymal stem cells have been studied; however, less attention has been paid to the drug metabolizing ability of these *in vitro* differentiated cells. The aim of this study was to evaluate the effect of laminin coating on CYP450 enzyme activity in our differentiated BM-MSCs.
Materials and Methods: The characteristics of MSCs obtained from Royan 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. On day 21, "P450 CYP3A4 screening system" was used to study the enzymativ activity. This method was a luminescence assay in which a CYP substrate converted by CYP enzymes to a luciferin product that in turn reacted with a Luciferin Detection Reagent to produce light. The amount of light produced was proportional to the amount of luciferin produced after the CYP reaction. Undifferentiated human MSCs were used as negative control.
Results: The flowcytometry findings revealed that over 90% of the cells expressed the MSC markers and not hematopoietic and leukocyte markers. The results showed that the activity of CYP3A4 was two times higher in the cells differentiated on laminin-coated vessels Compared to that of the Polystyrene and the differences were Significant statistically ($p < 0.05$). Increasing activity of this enzyme showed improved differentiation in laminin matrix. Furthermore, the results of P450 CYP3A4 screening system demonstrated that this enzyme had no activity in the human BM-MSCs before differentiation.
Conclusion: Together, these findings may indicate that laminin coating can improve the differentiation of hepatocyte-like cells from human BM-MSCs, at least in part by stimulating their CYP450 enzyme activity, which is fundamental for their drug detoxification function.
Keywords: CYP450, Enzyme, Hepatocyte, Laminin, MSCs

Ps-55: Hepatocyte-Like Cells Derived Human Endometrial Stem Cells An Ideal Candidate for Liver Regeneration

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Objective: The registered clinical application base on stem cell technology has been considered at least a close to 10 years due to some restrictions such as the lack of suitable cell source and the risk of teratoma formation. The current study aims to introduce human endometrial stem cells (hEnSCs) as adult stem cells with the easy access source and no immunological response, for alternative cell therapy.
Materials and Methods: The hEnSC were exposed to hepatojenic induction medium for 30 days and hepatic differentiation was assess via quantitative reverse transcription-polymerase chain reaction, immunofluresent staining for hepatic-specific genes and proteins, including cytokeratin 18, alpha-fetoprotein (AFP) and albumin (Alb) as well as functional tests for glycogen storage was performed.
Results: Cultured cells on hepatogenic medium differentiated into hepatocyte-like cells and expressed hepatic specific markers on day 30 of differentiation. Periodic Acid-Schiff Staining showed that hEnSCs were able to storage of glycogen.
Conclusion: In the presence study, the differentiated cells features were closely resembled human adult hepatocytes by *in vitro* testing. These cells will be provided possible sources of cells for liver repopulation.
Keywords: Human Endometrial Stem Cells, Hepatocyte-like Cells, Differentiation, Liver Regeneration

Ps-56: Evaluation of Effect of Tail and Lung Tissue Extract on Differentiation of MSCs to Nerve Cells

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Objective: Mesenchymal stem cells (MSCS) are important adult stem cells that attracted many researchers. Bone marrow derived MSCS have capable of differentiation to much kind of cells including fat cells, Osteocyte, Chondrocyte and Neuron. Most of substances that up to now used to induction of differentiation of stem cells to neural cells is: Retinoic acid, Dimethyl Solfuxid, Deprenil and ... that are toxic compounds or expensive. In this study, lung and tail tissue extracts of embryo is used as non-toxic inducing molecule.
Materials and Methods: MSCS prepared and cultured from adult mouse femur (BALB / C). After four subcultures, lung and tail tissue extracts with density of 50%, 70% and 80% in group I and II treated for 3 days and 7 days respectively. In morphological study, Krezil violet staining was carried out and in flowcytometry study, Nestin and Tubulin antibodies used for assess of molecular character of neurons.
Results: The flowcytometric results showed that in group I, the Nestin marker expression level in contrast to control group decreased significantly ($p < 0.05$), but Tubulin marker expression increased significantly. In group II, the expression levels of Nestin and Tubulin markers increased significantly in contrast to control group ($p < 0.05$). Results of Morphological and flow-

cytometric study were in line.
Conclusion: Induction of MSCS with extracts of lung and tail tissue increased the differentiation of MSCS to neuron. This study showed that MSCs have the ability to differentiate to neurons *in vitro* and this induction depends on the type of inducer.
Keywords: MSCS, Neuronal Cells, Induction, Differentiation, Lung and Tail Extracts

Ps-57: The Potential Impact of Cell Separation Methods on The Functional Capabilities Of UCB Derived CD133+ Cells

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Objective: Umbilical cord blood (UCB) is a possible alternative to other sources for transplantation of hematopoietic progenitors. However, delayed platelet engraftment is an inherent problem with cord blood transplantation. The aim of this study was comparison of the potential effects of direct and/or indirect magnetic-activated cell sorting (MACS) methods on the functional properties of UCB-CD133+ cells.
Materials and Methods: CD133+ cells were isolated from UCB samples by direct and indirect MACS methods and cultured in liquid serum-free stem span in the presence of interleukins 3 and 6, stem cell factor, and thrombopoietin. The cultures were then assayed for cell expansion and differentiation. In addition, colony-forming unit assay was performed using CD133+ cultures in serum-free collagen-based medium containing the cytokine cocktail. Colony forming unit megakaryocyte (CFU-MKs) and non-MKs were counted after immunocytochemistry staining on day 12.
Results: The number of the selected UCB CD133+ cells was higher in indirect than direct MACS ($p < 0.05$). Cells separated by indirect MACS had a significantly higher proliferative potential than direct method ($p < 0.05$). Expression rates of CD41, CD61, and CD42b were relatively higher in the direct- than indirect- derived cells on day 7, but the amount of increase was not statistically significant ($p > 0.05$). Interestingly, while the number of large colonies increased dramatically (about 54%) in the indirect-derived megakaryocyte (MK) progenitors, approximately 63% of the direct-derived MK colonies had small sizes.
Conclusion: It is probable that indirect-derived MKs with larger colony size would be more efficient in platelet engraftment and shortening the period of post-transplantation thrombocytopenia by producing higher platelet numbers.
Keywords: Umbilical Cord Blood, CD133+ Cells, Direct MACS, Indirect MACS, Megakaryocyte

Ps-58: The Antioxidative Effects of Soybean on Embryonic Chondrogenesis

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Objective: Free radicals such as reactive oxygen species are formed during a variety of biochemical reactions and cellular functions. Oxidative stress is responsible for a variety of degenerative processes in some human diseases. In aerobic cells, free radicals are constantly produced mostly as reactive oxygen species. Once produced, free radicals are removed by antioxidant defenses including enzyme catalase, glutathione peroxidase, and superoxide dismutase. Oxidative stress has been implicated in the pathogenesis of various diseases affecting chondrogenesis or the function of articular cartilage. DNA damage caused by oxidative stress may trigger the activation of the nuclear enzyme, poly (ADP-ribose) polymerase-1 (PARP-1) which may contribute to tissue injury. It has been demonstrate that increasing ROS levels induce chondrocyte hypertrophy. Reactive oxygen species (ROS) have been implicated in cellular damage; however, the physiological role of ROS in chondrogenesis is not well characterized. Diet can ameliorate oxidative stress. Diet has been known for years to play a key role as a risk factor for chronic diseases. Food contains many chemicals that have various effects on biological phenomenon. Some phytochemicals may contain anti-oxidative agents. It has been demonstrated that the incidence of the different type of cancer is less in ascians than the other nations and it can be related to using soybean in their diet. Soybean contains phytoestrogen, oxygenase-1 and tocopherol. It has been demonstrated that they play a protective role for mammalian cells against oxidative stress. The soy extract and its fractions displayed a strong free radical scavenger activity. It has been demonstrated that soybean extract impact on embryo chondrogenesis and ossification *in vivo* (JAAR). Many researches revealed that soybean has beneficial effects on cartilage. There is evidence that flavonoids and in particular isoflavones, may exert positive effects on cartilage metabolism and skeletal health. Flavenoid and isoflavenoid are the phytoestrogen and soybean is rich source of these components. There are many investigations that focus on the mechanism of soybean effects. Some of them focus on the its anti-inflammatory effects and the other mentioned its antiestrogenic or estrogenic properties With regards to these considerations, the Objective of this project will be to find the impact of soybean extract on reduction of detoriation effects of oxidative stress in embryonic chondrogenesis *in vitro*.
Materials and Methods: 12-13 days old mice embryos

used to extract mesenchymal cells. For this purpose, the limb bud of embryos removed and after rinsing in PBS containing antibiotic, incubated in dispase for 90 minutes in order to separate ectoderm from mesenchyme. After separation, the limb bud rinse and incubate in the trypsin for 20 minutes. The limb bud became dissociation by adding culture media (DMEM/F12 contain 10% FAS, 2 mM glutamine and antibiotic) and pipetting. The cell was count by hemocytometer and the number of the cells adjusts to 1-2 $\times$ 10^5. Superconfluent micromass culture of limb bud mesenchyme established by spotting 10 µl of cell suspention in 96 well tissue culture plate. After incubation for 1.5-2 hours at 37 ºC and 5% CO_2 to permit cell attachment, the plate will be flooded with culture media. The cells allowed to confluent for 4 days. The culture media changed daily. Some wells stained with alcian blue to improve that the cartilage was differentiated. At day 5 first, the sample divided to 10 groups and different concentration of H_2O_2 and soybean separately and mixed with each other added to fresh media and media without any soybean extract as negative control and incubated for 24 hours in order to induce oxidative stress. The day after that, the medium changed by culture media (DMEM/F12 contain 10% FAS, 2 mM glutamine and antibiotic) without any soybean extract as negative control added to the wells. Each group will be done in triplicate. Each well stained with alcian blue and the size and the number of the nodules calculated.

Results: The viability rates of each group were separately compared to control and the results showed that there were significant different between each experimental group as compared to control ($p \leq 0.05$). The best result was for the group that the soybean was mixed with the 1000 µm concentration of H_2O_2. We also detected that the high concentration of soybean reduced the viability. So between the different concentrations of soybean the lowest concentration (10 µm) was the best.

Conclusion: It has been demonstrated that soybean extract impact on embryo chondrogenesis.Flavenoid and isoflavenoid were the phytoestrogen and soybean was rich source of these components.we found the impact of soybean extract on reduction of detoriation effects of oxidative stress in embryonic chondrogenesis in vitro.

Keywords: Limb Bud, Chondrogenesis, Antioxidant, Soybean

Ps-59: Derivation of Oligodendrocyte Progenitor Cells (OPC) from Embryonic Stem Cells (ESCs) Generated by Somatic Cell Nuclear Transfer

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Objective: Spinal cord injury can lead to death of oligodendrocytes and axonal demyelination. It has been shown that mature oligodendrocytes are incapable of repair the demyelination after injury to myelin and oligodendrocyte progenitor cells (OPCs) have capacity of myelin repair. Studies demonstrated that they must be differentiated to the myelinating cells in order to result in remyelination. In this study, we examined differentiating potential of mouse embryonic stem cells (ESCs) generated through somatic cell nuclear transfer into oligodendrocyte progenitor cells.

Materials and Methods: In this research, we used S1 nuclear transfer embryonic stem cell (NTESc), GFP+ gifted from stem cell technology research center. NTESc derived from this colony were cultured on inactivated mouse embryo fibroblasts (MEFs) as a feeder layer. After forming embryoid bodies, those EBs are disaggregated and plated on OPC medium culture containing of retinoic acid for 4 days. Gene expression evaluated by Real Time PCR and flowcytometry.

Results: Embryoid bodies formed from these colonies. The purity of oligodendrocyte progenitor cell (OPCs) was high. The oligodendrocyte progenitor cell markers (Olig2, NG2) were increased and a decrease in expression of mature oligodendrocyte marker (MBP) was observed.

Conclusion: We found that NTESC that are produced by therapeutic cloning have high differentiating ability to produce oligodendrocyte progenitor cells Therefore, this technique may facilitate maintenance, culture and differentiation of stem cells and OPC produced through this way might be used to reconstruct injury site. It has been considered as an efficient therapeutic strategy in spinal cord injury.

Keywords: Somatic Cell Nuclear Transfer, Embryonic Stem Cells, Oligodendrocyte, Myelin Repair, Spinal Cord Injury

Ps-60: Evaluation of MicroRNA-16 Functions in Breast Cancer

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Objective: MiR-16 plays a critical role as a tumor suppressor by targeting several oncogenes such as genes controlled cell cycle and apoptosis. Hence, miR-16 could act as an important anti-tumor agent in most cancers. In the present study, we investigated the functional role of miR-16 on breast cancer growth and survival.
Materials and Methods: Human breast cancer cell line, MCF-7, was infected with lentiviruses containing miR-16 precursor sequence. The effects of ectopic expression of miR-16 on breast cancer phenotype were examined by cell cycle and apoptotic assays. miR-16 cytotoxicity was measured by the MTT assay.
Results: We showed that the miR-16 over expression reduces CyclinD1 mRNA and Bcl2 protein levels. In addition, we found that enforced expression of miR-16 declined cell growth, proliferation and induces apoptosis.
Conclusion: Taken together, our results revealed that the up-regulation of miR-16 would be a potential approach for breast cancer therapy that induces G1 arrest and apoptosis.
Keywords: MicroRNA, miR-16, MCF-7, Apoptosis, Cell Cycle, Breast Cancer, Cancer Therapy

Ps-61: Down-Regulation of The Genes Involved in Reprogramming (OCT4, SOX2, P21, ESC-specific microRNA miR-302 and miR-145) in Gastric Adenocarcinoma Reveals New Insight into Gastric Cancer

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Objective: Induced expression of a defined number of embryonic stem cell (ESCs)-specific genes, mainly OCT4 and SOX2, could reprogram a differentiated cell into an induced pluripotency stem (IPS) cell. The program is also inhibited by the action of some tumor suppressor genes, most importantly P53. There is a possibility that the re-expression of ips genes, i.e. OCT-4 and Sox2, and diminished expression of p53 and its main down-stream targets, i.e. P21 and miR-145, contributes also to tumorigenicity.
Materials and Methods: A total number of 34 tumor and their matched non-tumor (as control) gastric surgical specimens were obtained. The expression and tissue distribution of the genes of interest were evaluated by means of real-time PCR and immunohistochemistry (IHC).The human embryonic carcinoma cell line, NTERA2 (NT2) and a human gastric adenocarcinoma cell line, AGS, were used as controls.
Results: Our data revealed a significant down-regulation of P21 and miR-145 genes in tumor vs. non-tumor samples. The same finding was obtained for OCT4 variants, miR-302 and SOX2 expression in high-grade tumors. The ability of genes to discriminate tumor from non-tumor gastric samples was evaluated using the area under the receiver operating characteristic (ROC) curve. SOX2 showed the highest total area under the curve (AUC) (79%, p = 0.000). Although it found that the AUC for combination of miR-145 and p21 (71%, p=0.004) and all variants of OCT4 (75%, p = 0.008) has a better discrimination power than using each ones individually. In coordination to real-time PCR data, the results of immunohistochemistry for Sox2 protein showed the expression of Sox2 protein in tumor and non-tumor samples. Interestingly, despite the confirmation of OCT4A transcript expression in gastric tumor samples, we failed to detect any nuclear localization signal for Oct4 A protein in different grades of gastric tissue samples.
Conclusion: Despite the fact that some hESC-specific genes are up-regulated in tumors, our data revealed down-regulation of miR-302b, OCT4 and SOX2 in high-grade tumors. So it suggests a specific regulatory role for these genes in the cell cycle control in the gastric samples. We also found that the expression of all genes are significantly vary between diffuse vs. intestinal as well as in high grade vs. low grade types of gastric tumor samples. Based on ROC curves, it seems that evaluation the expression of SOX2 individually and also miR-145 and P21 together are informative diagnostic tools for gastric tumors.
Keywords: Reprogramming, IPS, Oct4, SOX2, MiR-302, P21, MiR-145, MiR-302b, Gastric Cancer

Ps-62: miR-302b, an ESC-specific MicroRNA, is Down-Regulated in Gastric Adenocarcinoma

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Objective: microRNAs (miRNAs) are a new class of non-coding RNAs involved in regulating various biological processes including proliferation, differentiation, and apoptosis, among others. Alterations in miRNA expression are reported in several human cancers, which suggest their potential roles in tumor initiation and progression. Members of the miR-302 cluster are

highly expressed in embryonic stem cells (ESCs), where they regulate cell self-renewal and pluripotency. Based on the cancer stem cell (CSC) hypothesis, mis-expression of such genes might contribute to tumorigenicity. This study aims to find a potential link between the expression level of human/homo sapiens miR-302b (has-miR- 302b) and tumor/grade state of gastric tissues.
Materials and Methods: A matched based case-control study was conducted that included tumor and matched marginal non-tumor surgical specimens from 34 patients diagnosed with gastric adenocarcinoma. Real-time reverse transcriptionpolymerase chain reaction (RT-PCR) assays were performed with specific LNA™ primers and SYBR Green master mix. The human embryonic carcinoma cell line, NTERA2 (NT2) and a human gastric adenocarcinoma cell line, AGS, were used as controls. The ability of miR-302b to discriminate tumor from non-tumor gastric samples was evaluated using the area under the receiver operating characteristic (ROC) curve.
Results: According to our data, miR-302b expression (normalized to that of the U6 snRNA housekeeping gene) in the pluripotent cell line NT2 was more than 500 times greater than that of the AGS cell line. The level of expression was even lower in tumor and non-tumor gastric tissue samples. The data further revealed a down-regulation of miR-302b in gastric tumor samples (p=0.001), particularly in high-grade adenocarcinoma (p=0.009). However, ROC analysis data demonstrated a low sensitivity and specificity of miR-302b expression to discriminate between the tumor and non-tumor state of the samples (AUC=0.63).
Conclusion: Despite the upregulation of some hESC-specific genes in tumors, our data revealed a down-regulation of miR-302b in high-grade tumors. This data suggested a potential tumor-suppressor role for miR-302b in tumorigenesis of gastric tissue.
Keywords: Cancer Stem Cells, MicroRNA, MiR-302b, Gastric

Ps-63: A Three-Dimensional Fibrin Scaffold Promotes Neuronal Differentiation of PC12 and Embryonic Stem Cells while Reducing Apoptosis

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Objective: The current study was aimed to compare the quality of neuronal differentiation and reduction in apoptosis that occurred in two-dimensional (2D) and three-dimensional (3D) fibrin scaffold culture conditions. PC12 and embryonic stem cells are two commonly utilized cell lines for the study of neuronal regeneration.
Materials and Methods: These cells were induced to neuronally differentiate by adding NGF and retinoic acid respectively. Total neurite length and expression of neuronal markers (MAP-2 and β3-tubulin) was assessed by morphometry and immunocytochemistry. Also, TUNEL assay was used to detect apoptosis.
Results: Upon exposure to a differentiation media in the 3D fibrin gel, PC12 and embryonic stem cells stopped dividing, had increased adhesion to the substratum, extended neurite processes and expressed neuronal markers. The same results, however, were not observed with the 2D culture. Also, the apoptosis index performed by TUNEL demonstrated a reduction in the degree of apoptosis in the 3D culture compared to 2D culture (p =0.001). 3D fibrin scaffolds support growth and neuronal differentiation of PC12 and embryonic stem cells. In addition, the 3D culture enhanced cellular resistance to apoptosis when compared to the 2D culture.
Conclusion: It appears as if a 3D culture system may offer a better technique for future neuronal tissue engineering investigations.
Keywords: 3D Culture, Apoptosis, Fibrin Gel, Embryonic Stem Cells, PC12 Cells

Ps-64: Can Human Eye-Derived Induced Pluripotent Stem (iPS) Cells Efficiently be Programmed to Oligodendrocytes ?

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Objective: Oligodendrocytes are myelinating cells in the central nervous system (CNS) that form the myelin sheath of axons to support rapid nerve conduction. The technology to generate autologous pluripotent stem cells (iPS cells) from almost any somatic cell type has brought many hopes to cell replasment therapy of neurodegenerative diseases.
Materials and Methods: In this study iPS cells were directly generated from the human conjunctive-stromal cells overexpression of Oct4, sox2, C-myc and KLF4 transcription factors. For oligodendrocyte differentiation, iPSCs were cultured in N2 medium supplemented with FGF2, EGF, PDGF-AA (20 ng/ml) and T3 (30 ng/ml) for 21days. Differentiated cells were analyzed for expression of oligodendrocytic cell markers such as Nestin, Olig2, PDGFRa, CNP in the level of mRNA by quantitative RT-PCR. To confirm the expression of the marker in the level of protein the ICC for some markers such as olig2, A2B5, PDGFRa were done.
Results: The results of ICC and qRT-PCR show that human iPS-derived oligodendrocyte precursor was positive for oligodendrocyte markers.

Conclusion: The *in vitro* differentiation of human eye-derived iPS cells to oligodendrocytes may provide new perspectives resource cell replacment therapy of neurodegenerative diseases.

Keywords: iPSCs, Oligodendrocyte, Differentiation

Ps-65: Mesenchymal Stem Cell Conditioned Medium Accelerates Regeneration of Human Renal Proximal Tubule Epithelial Cells after Gentamicin Toxicity

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Objective: Bone marrow-derived mesenchymal stem cells (BM-MSCs) have the capacity to regenerate renal tubule epithelia and repair renal function without fusing with resident tubular cells. The goal of the present project was to investigate the role of BM-MSCs secreted cytokines on tubule cell viability, differentiation, epithelial-to-mesenchymal transition (EMT) and regeneration.

Materials and Methods: We used a conditionally immortalized human proximal tubule epithelial cell line (ciPTECs) and the nephrotoxic agents, gentamicin and cisplatin to induce EMT. Cell viability of ciPTECs was be measured by an MTT assay. The process of EMT was evaluated by determining the induction in vimentin expression by fluorescence-activated cell sorting (FACS) analysis. Finally, migration of PTECs were be studied by using a scratch assay.

Results: A significant increase in cell death was observed with increasing gentamicin or cisplatin concentrations. As cisplatin is a powerful toxicant for tubular cells it caused necrosis at high concentrations. Our results showed a significant increase of vimentin-postive cells after exposure to gentamicin or cisplatin compared to the medium control, indicating a process of EMT and mesenchymal differentiation in ciPTEC. However, the increase of vimentin expression at high concentrations of cisplatin was related to morphology transformation and cell necrosis. ciPTECs exposed to cisplatin followed by exposure to fresh medium did not result in recovery. However, ciPTECs exposed to high doses of gentamicin followed by a recovery period in fresh medium, resulted in a significant increase in cell migration and cell transformation. This effect was accelerated when ciPTECs were exposed to human mesenchymal stem cells conditioned medium (hMSCs-CM) after high doses of gentamicin. In addition, hMSCs-CM caused a recovery and increased cell viability of cells exposed to gentamicin.

Conclusion: High doses of gentamicin and multiple days dosing caused an increase in cell death and a lack of ability to migrate into a scratch-wounded area. We further demonstrated this effect was recovered partially by exposing cells hMSCs-CM. Our data suggest that MSC secreted cytokines stimulate renal tubule cell regeneration after nephrotoxicity.

Keywords: Conditionally Immortalized Human Proximal Tubule Epithelial Cell (ciPTEC), Human Mesenchymal Stem Cells Conditioned Medium (hMSCs-CM), Gentamicin, Nephrotoxicity, Regeneration

Ps-66: Isolation and Characterization of Prostate Cancer Stem-Like Cells from PC3 Cell Line

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Objective: Prostate cancer has become the most frequently diagnosed cancer and the second leading cause of cancer-related deaths in North American men. There is now increasing evidence in some malignancies including prostate cancer that the tumor cells are organized as a hierarchy originating from rare stem cells that are responsible for maintaining the tumor and its metastasis to other organs resulting to a malignant state in cancer. Different markers including CD44, α2β1and CD133 are expressed in these cells. The aim of this study is the isolation of CD44 positive cells and characterization of stem cell properties of positive and negative sorted cells.

Materials and Methods: PC3 cells were cultured on complete medium. Cells were sorted as 2 different groups; CD44+/CD49b^{high} (cancer stem-like cell) and CD44-/CD49b^{low} (non cancer stem cell) using BD FACS Aria cell sorter. Colony formation assay, spheroid assay and doubling time were done on sorted cells. qRT-PCR was used to compare expression of CDH1, Sox2, Oct4 and Nanog genes between 2 groups.

Results: Sorted cells formed 3 different phenotypic of colonies named holo, mero and para colon. The number of holo and mero colon were higher in CD44+/CD49b^{high} cells compared to CD44-/CD49b^{low} group (27.15% vs. 25% and 26.75 vs. 2.81% subsequently) whereas the number of para colons were high-

er in CD44- cells (4.12% vs. 2.18%). CD44+ cells were also more capable to form spheroids in serum free media than CD44- cells which correlate to doubling time for CD44+ and CD44- cells. The CDH1 and Stemness related genes were expressed in higer level in positive group.
Conclusion: Our data reveal that prostate CSCs are a subpopulation of CD44+ cells within the tumor. These cells have a high capacity to form holo and mero colons and have high proliferation rate .The reason of high expression of CDH1 in these cells remained as a question in this study and needs more investigations.
Keywords: Prostate Cancer, Cancer Stem Cell, CD44, CD49b

Ps-67: Germ Cell Proliferation under Several Culture Conditions

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Objective: In this study we compare the *in vitro* effect of retinoic acid (RA), on the ESCs- derived primordial germ cells (PGCs) proliferation.
Materials and Methods: CCE mouse ESCs were cultured for 1 day in order to embryoid body (EB) formation and then cultured for 4 days in the presence of 5 ng/ml BMP4 for PGC induction. In order to PGC enrichment, ESCs derived germ cells cultured for 7 days in the presence or absence of 3 μM RA in the STO co-culture system. Expression of Mvh protein, the PGC specific marker, was evaluated immunocytochemically. Data analyses were done with ANOVA and Tukey posttest.
Results: The results of immunocytochemistry showed that the mean percentage of immunostaining cells of Mvh was increased significantly in RA-treated cells compared with RA free group, BMP4-treated group, BMP4 free group, 1-day-old EB and ESCs.
Conclusion: The results suggest that RA is an efficient inducer in PGC enrichment from mouse ESC.
Keywords: Embryonic Stem Cell, RA, Germ Cell, Mvh

Ps-68: The Best Mesenchymal Stem Cell-Conditioned Medium for Wound Healing

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Objective: Skin undergoes through continuous expansion and renewal through life. It loses its integrity in many ways. Keeping skin integrity has special value. Mesenchymal stem cells are useful for wound healing and skin repair. Recently, mesenchymal stem cells-conditioned mediums are discovered to have almost the same beneficiary effect on skin, but conditioned medium of which source has the best wound healing effect remained to be investigated. The purpose of this study was to compare the effect of conditioned medium from three different sources of mesenchymal stem cells in wound healing.
Materials and Methods: Fibroblasts were treated with adipose derived stem cell-conditioned medium (ADSC-CM), bone marrow mesenchymal stem cell-conditioned medium (BMSC-CM) and umbilical cord blood stem cell-conditioned medium (UCBSC-CM) and DMEM/F12 with 10% FBS as a control group. Gene expressions of hyaluronan (HA), collagen type I, fibronectin and secreted amount of HA, fibronectin and collagen I were measured. Fibroblast migration and proliferation were also assessed.
Results: The difference of some gene expressions between the treated groups and the control were significant but they were not very obvious. The secreted amount of HA, fibronectin and collagen was at highest point in the group of fibroblasts treated with ADSC-CM. Fibroblast migration rate was at the fastest point in the control group containing 10% FBS and then in the group treated with UCBSC-CM. No significant difference between the proliferations of 4 groups was observed.
Conclusion: Among mesenchymal stem cells, ADSC have more stimulatory effect on fibroblasts to secret more ECM components and that could be due to the difference of amount or type of secreted growth factors and cytokines from it.
Keywords: Fibroblast, Wound Healing, Mesenchymal Stem Cells, Conditioned Medium

Ps-69: RGD Modified PCL/Gelatin Hybrid Nanofiber for Potential Applications in Tissue Engineering

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Objective: Nanofibrous polycaprolactone (PCL) scaffolds have many advantages in biomedical applicatipns,

but its usefulness in tissue engineering is limited because of the lack of bioactivity. In this work, we developed a PCL/Gelatin hybrid nanofibrous scaffold which further functionalized by RGD-containing peptide as a biomimetic cell adhesion motif.
Materials and Methods: Collagen-derived RGD-containing peptide with an Iodine tag was synthesized using standard Fmoc-chemistry on Rinkamide-MBHA resin. The peptide was cleaved by 95% TFA, precipitated by diethyl ether, lyophilized, and purified by Preparative HPLC. MALDI-TOF mass spectrometry was used for analyzing peptide mass. A solution of 10% (w/v) PCL and Gelatin (1:1) was dissolved in trifluoroethanol, then PCL/Gelatin nanofibrous scaffold was fabricated by electrospinning. To improve performance and structural stability, electrospun scaffold was crosslinked by EDC. To develop hybrid, bioactive scaffold, RGD-containing peptide was immobilized on activated scaffold by the same strategy as crosslinking. Immobilization of peptide was assessed by UV absorbance of the peptide. The morphology of nanofibers was characterized by SEM.
Results: Successful peptide synthesis was confirmed by MALDI-TOF mass spectrometry with theoretically predicted mass of 1185.21. SEM images verified PCL/Gelatin nanofiber fabrication with diameters between 300 to 400 nm which appeared in random network. SEM images showed successful crosslinking of PCL/Gelatin nanofibers. UV spectrophotometry results indicated that the peptide has been immobilized on scaffold with a concentration of about 200 $\mu g/cm^2$.
Conclusion: The present study introduces a hybrid nanofiber scaffold that further functionalized by immobilization of RGD-containing peptide. This RGD-functionalized PCL/Gelatin nanofiber scaffold improves cell-biomaterials interaction and could have potential applications in tissue engineering and regenerative medicine.
Keywords: PCL, Gelatin, RGD, Scaffold, Nanofiber

Ps-70: Osteopontin, As An *In Vitro* Survival Factor for Treating Damage of Human Oligodendrocyte Progenitor Cells under ROS Induced Conditions (h-OPCs)

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Objective: In neurological disorders including MS, oligodendrocytes and oligodendrocyte progenitor cells (OPCs) damages and demyelination are associated with a considerable degeneration of axons. Reactive oxygen species (ROS) such as H_2O_2, are one of the main apoptotic factors that cause oxidative stress and death of OPCs. Remyelination is generative event in which demyelinated axons are reinvested with new myelin sheaths via interactions between OPCs and demyelinated axons. There is strong evidence that extracellular matrix (ECM)-integrin interactions are potent regulators of OPCs, survival and migration. Furthermore, Osteopontin (OPN) as an ECM ligand, is expressed at high levels in demyelination lesions. We have examined the exogenous recombinant human OPN(hrOPN) effect on survival of human OPCs culture under ROS induced condition.
Materials and Methods: rhOPN diluted in PBS and coated onto 96-well plate in a defined serial concentration (12, 50, 100, 200, 400, 800 ng/ml). After 30 minutes incubation at 37°C, ECM was removed and hOPCs were plated at a density of 5000 cells/well. The medium solution in each well was removed after 24 hours and cells were treated with serum free medium that contained an optimum concentration of H_2O_2 which induced apoptosis in OPCs. The cell were incubated for 24 hours and MTS solution was added to each well for cell viability assay. After 4 hours incubation at 37ºC, optical densities (A) at 492 nm were measured using an ELISA plate reader.
Results: MTS results showed that OPCs exhibited increasing apoptotic rates in response to treatment with H_2O_2 compared to untreated cells. In contrast OPCs plated on Osteopontin-coated wells showed a minimal rate of cell death after H_2O_2 treatment.
Conclusion: H_2O_2 induced loss of cell viability in human OPCs, however rhOPN coating on culture dish, significantly suppressed apoptosis and enhanced cell viability of OPCs considerably. Thus we concluded that OPN is an appropriate survival factor for OPCs culture under ROS induced condition.
Keywords: Osteopontin, Remyelination, Reactive Oxygen Species, Oligodendrocyte Progenitor Cells, Apoptosis

Ps-71: Assessment of Methamphetamine-Induced Neurotoxicity on Embryonic Stem Cell-Derived Neural Cell

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Objective: The neurotoxic amphetamines (especially methamphetamine [METH]) are among the most widely-abused drugs. This drug has even higher ratio of entering to nervous system in comparison to other amphetamines. It is well established that these compound

are neurotoxic both *in vivo* and *in vitro*. This in turn gives rise to concern that the fetus may be at risk when carried by an MA user unaware of being pregnant, but there have been no long term studies of the neurodevelopment consequences of prenatal MA in humans. There is an essential need to incorporate embryotoxicity tests on the embryonic period into standard screening models for appraising effects of pharmacological agents. In recent decades undifferentiated pluripotent embryo-derived stem cell lines which resemble early embryonic stages to analyze toxic effect of components *in vitro*. Thus, this study aimed to assess toxic effects of MA on embryonic stem cell (ESC)-derived neuronal cells during differentiation in pharmacological model.

Materials and Methods: ESC line Royan which was derived from the C57BL/6 mouse strain was used throughout this study. ESC-derived neural cells were treated with different concentrations of MA. The effect of MA on neural differentiation was assessed during two periods: group 1: MA was added during embryoid body (EB) formation until day 10, group 2: MA was added from day 6, after RA induction or the generation of neural precursors until day 10. In addition, Four days after EB plating, the cells were prepared for evaluating microtubule associated protein 2 (MAP2) and Nestin expression with immunocytochemistry and in RT-PCR these gene expressions were evaluated with different concentrations of drugs.

Results: Morphological studying of RA-treated EBs by immunostaining showed the presence of neural outgrowth in the absence of MA while any neural outgrowths were not observed in presence of MA. We assessed ID 50 (inhibit differentiation) 130 and 400 μM in group1 and 2, respectively. Moreover, results of semi-quantitative RT-PCR at four days post-plating showed, in spite of no significant change in Nestin expression, MAP2,as an important cytoskeletal protein, was significantly reduced in concentrations greater than 200 and 500 μM in groups 1 and 2. Thus, comparison between morphological studies and RT-PCR stated that there is closure correlation among structural changes with alterations in gene expression and could be resulted that MAPs are involved in the preservation of mature neuronal morphology, neurite outgrowth and neuronal plasticity.

Conclusion: MA was resulted in a marked reduction in neural markers in ESC cultures and these toxic effects can be augmented when being used early embryonic development and engender deteriorate influence on neuronal integrity by changing the cytoskeletal assembly. Also, these data confirmed that in neural differentiation, the progenitor phase is more sensitive than mature phase. According to this current experiment and our previous study, we propose that embryonic stem cells are suitable model for assessment of drug toxicity, especially when assessment of drug *in vivo* is difficult and large number of animal are required to be sacrificed to achieve similar conclusion.

Keywords: Methamphetamine, Embryonic Stem Cell, Neural Differentiation, Toxicity

Ps-72: Evidence for Expression of ID Gene Family Members in Human Embryonal Carcinoma Cells

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Objective: The basic helix-loop-helix (bHLH) family of transcription factors function in the coordinated regulation of gene expression, through different cellular processes such as cell lineage commitment, embryogenesis/organogenesis, tumor progression, and so on. Helix-loop-helix ID (Inhibitor of DNA binding) proteins are distinct from other bHLH transcription factors in that they lack the basic domain necessary for DNA binding. ID proteins thus function as dominant negative regulators of bHLH transcription factors. The inhibition of bHLH factor activity by forced constitutive expression of ID proteins is closely associated with the inhibition of differentiation in a number of different cell types, and it is supposed that various members of the ID family have active roles in embryonic stem cells self-renewal and also in a range of human tumors. Embryonal carcinoma (EC) cells derived from testicular tumors are valuable cellular model systems for investigating embryogenesis and developmental biology processes. Since EC cells are malignant but their terminally differentiated derivatives are not, understanding the expression profile of these embryonal cells may be of value for diagnostic and maybe therapeutic purposes in embryology and development.

Materials and Methods: In the current work, the mRNA expression levels of the all four ID gene family members were quantitatively evaluated by RT-PCR technique, in a human embryonal carcinoma cell line named NT2/NTERA2.

Results: The results declared significant expression levels of ID genes in embryonal carcinoma cells.

Conclusion: This finding confirms the dynamic role of these transcription factors in embryogenesis and carcinogenesis.

Keywords: ID Genes, NT2/NTERA2, Embryogenesis, Carcinogenesis

Ps-73: Long Term Storage and Banking of Stem cells Exist in Peripheral Blood

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Objective: Freezing the peripheral cells is suitable therapeutic method and approach for long term storing to the patients suffering from cancer such as Multiple myeloma, Hodgkin's lymphoma, Non-Hodgkin's lymphoma and after the chemical therapy. This study presents the result of storing and transplantation of peripheral blood cells after four years.
Materials and Methods: Donors were patients or their close relatives. Intravenous GCST 300 Mg/Kg (Neupogen) were injected for five days. Increasing of hematopoitic stem cells were followed by CBC count, then samples sent to Royan public cord blood bank. The number of cells stored in the bag was $6\times10^9 \pm 1$. DMSO and DEXTRAN were used for freezing the peripheral samples. The number of cells, percentage of viability, percentage of CD34+, number of colonies and their types were evaluated before and after freezing.
Results: 40 blood units were stored from 2008 to 2011. The average number of nucleolus cells (NC) was $66.1\times10^9 \pm 29.95$. The number of CD34 cells was $20.24 \times 10^9 \pm 11.69$ and CFC was 57.38×10^4. The result of melting three samples after one year being in frozen state showed that the number of living cells had decreased by living cells. In the meanwhile, the function of CD34+ cells does not change significantly.
Conclusion: Transplantation on three patient suffering from uterine cancer, Hodgkin·s lymphoma, Non-Hodgkin's lymphoma, were successful and present while more than two years have passed, no disease return have occurred.
Keywords: Peripheral Blood Stem Cells, Cellular Freeze, Colony Appraisal Unit, Peripheral Blood Bank

Ps-74: Developmental properties of The Primordial Germ Cells in Sheep Fetus

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Objective: The genital ridge is also invaded by primordial germ cells (future spermatogonia or oogonia) that are known as vegetative cells or somatic cells. PGCs originated from the yolk sac and after colonizing in gonads, give rise to gametes that are responsible for the development of a new organism in the next generation. The purpose of this study was to determine morphologic and histometric properties of the PGCs in sheep fetal testis.
Materials and Methods: Nine sheep fetuses were collected from slaughterhouse in the Shahrekord district and were taken to the embryology laboratory. After measuring crown-rump length (CRL) of fetuses, samples were divided into three age groups.
Results: In all of the groups, the microscopic studies revealed that primordial germ cells was seen spherical euchromatin nucleus with one or two nucleolus (es) that with increasing age got more euchromatin. In gonocyte cytoplasm was seen vacuolated structures with differences sizes in all ages. Morphometric studies also exposed that diameter of cells in second group were increased more than other groups, but were not significant ($p>0.05$). The most amount of the nucleus diameter was observed in third group and the least amount of the nucleus diameter was seen in second group, but these differences were not statistically significant ($p>0.05$). The number of PGCs in all aging groups also were not significant ($p>0.05$).
Conclusion: Our results detected that in PGCs developmental process, there is a little changes in the morphologic and histometric properties.
Keywords: Sheep, Testis, Primordial Germ Cells, Development

Ps-75: Generation and Maintenace of Human Embryonic Stem Cells-Derived Neural Precursors Cells by Small Molecules

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Objective: Human embryonic stem cells (hESCs) have led to an important revolution in stem cell research and regenerative medicine. In order to create specific neural progenitors (NPs), we have established a homogenous, expandable and self-renewable population of multipotent NPs from hESCs using an adherent system and defined medium supplemented with a combination of small molecules.
Materials and Methods: Here, after generation of NPs, we characterized them and evaluated their potency by Real time PCR, Immuno fluorecense and Flow cytometery satining, karyotype and pacth clamp analysis.
Results: The established hESCs-NPs highly expressed Nestin and Sox1 and Pax6. These NPs were continuously propagated for approximately six months without losing their potential to generate astrocytes, oligodendrocytes, and functional neurons, and maintained a stable chromosome number. Voltage clamp analysis revealed outward potassium currents in hESC-NPs. The self-renewal characteristic of the NPs was demonstrated by a symmetrical mode of Nestin-positive cell division. Additionally, these hiPSC-NPs can be easily frozen and thawed in the presence of ROCK inhibitor without losing their proliferation, karyotype stability, and developmental potential.
Conclusion: The characteristics of our generated hESC-NPs provide the opportunity to use patient-spe-

cific or ready-to-use hESC-NPs in future biomedical applications.
Keywords: Neural Prrecusor Cells, Human Emberyonic Stem Cell, Small Molecules

Ps-76: Regulation of Pancreatic Cancer Stem Cell Characteristics through P21

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Objective: p21 is a transcriptional target of p53 and is down-regulated in pancreatic cancer. This study aimed to investigate the functional significance of p21in pancreatic cancer progression through its epigenetic restoration with demethylating agent 5-Aza-29-deoxycytidine (5-Aza-dC).
Materials and Methods: Human pancreatic cancer stem cells(CSCs) (CD44+/CD24+/ESA+) have been characterized. CSCs were cultured in DMEM. Cell cycle distribution and of pancreatic cancer cells after treatment with Aza-5-dC and untreated controls were determined by flow cytometry DNA analysis Evaluation of mRNA expression levels was down by quantitative real time-PCR.
Results: Re-expression of p21in CSCs and in human pancreatic cancer cell lines upon treatment with 5-Aza-dC strongly inhibited the cell proliferation, cell cycle progression, self-renewal.
Conclusion: The present study thus demonstrates the role of p21 as a critical regulator of pancreatic cancer progression by the regulating CSC characteristics. The restoration of its expression by 5-Aza-dC will provide mechanistic insight and therapeutic targets for pancreatic cancer.
Keywords: Cancer Stem Cell, Pancreatic Cancer, p21, Methylation

Ps-77: Inhibition of SIRT1 Induces Apoptosis in Breast Cancer Stem Cells

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Objective: Over expression of SIRT1, is implicated in many cancers and therefore could become a promising antitumor target. Here we demonstrate a small molecule inhibitor, with potent inhibitory effects on the proliferation of breast cancer stem cells.
Materials and Methods: Breast cancer stem cells cultured in RPMI-1640 medium and treated with and without small molecule inhibitor. The percentage of apoptotic cells was measured by flow cytometry. Real-time quantitative RT-PCR was performed to quantitatively estimate the mRNA expression of Sirtuin1 at different times.
Results: Our findings indicated that small molecule inhibitor could effectively induced apoptosis in breast cancer stem cells. Further we showed expression of SIRT1 was dramatically down-regulated by small molecule inhibitor treatment with asending time.
Conclusion: Small molecule inhibitor as a single agent through decrease expression of Sirtuin1 gene induced significant apoptosis of breast cancer stem cells. So, the small molecule inhibitor may have constituted novel anticancer agent.
Keywords: Breast Cancer, Apoptosis, SIRT1, Stem Cells

Ps-78: Stem Cell Treatment for Childhood Absence Epilepsy

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Objective: Epilepsy is a brain disorder involving repeated and spontaneous seizures. Epilepsy is also called seizure disorder. A seizure occurs when a surge of electrical activity affects part of the brain. Seizures can have many symptoms and can last from a few seconds to several minutes. They are typically characterized by a loss of consciousness and convulsions. Childhood absence epilepsy (CAE) presents between ages 3 and 5 years and usually remits by ages 10-12 years. Childhood absence epilepsy is usually not associated with tonic-colonic seizures. Electroencephalography (EEG) shows a normal background for age and 3-Hz generalized spike and wave discharges. In this study we want to characterize the effect of stem cells for treatment the childhood absence epilepsy.
Materials and Methods: In this study we selected 12 children (2-6 years old) with a history of epilepsy for more than 6 month that treatment with stem cells implantation (stem cells contain NPY, an anticonvulsant agent that exists in the healthy human brain) in tow procedures, one of them for 7weeks, and the other on after 6 months. EEG, the symptoms of epilepsy and duration are observed and notated before and after stem cell therapy.
Results: In 9 patients seizures are much more under control, and 3 children only had seizures 5-10 times per day, and they last only 1-2 seconds. In all patients EEG was changed and generalized spike and wave discharges decreased.
Conclusion: Plasticity of embryonic stem cells can help to repair brain cells that have been damaged by repeated seizures. Seizure disorders are characterized by the hyper excitability of different types of brain cells, meaning they are more susceptible than healthy brain

cells to electrical storms
Keywords: Epilepsy, Absence, NPY

Ps-79: Study the Influence of IFN-g and/or LPS on Survival of Human Oligodendrocyte Precursor Cells

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Objective: IFN gamma is a pleiotrophic cytokine which is produced by a variety of immune cells in the development of disease such as multiple sclerosis. There are supporting evidences implying a role for NO in tissue damage during demyelinating diseases through LPS induction. Recent studies showed that the influence of IFN-g and/or LPS on survival and rat oligodendrocyte progenitor cells (OPCs) differentiation. In this study we investigated the effects of these factors on survival of human OPCs.
Materials and Methods: OPCs were grown for 1 day in Serum free medium were treated for 12 and 24 hours with IFN-g or a combination of LPS and IFN-g at different concentrations and survival of these cells was assayed utilizing MTS.
Results: As shown in data, LPS with 15 µg/ml and IFN-g with 100 ng/ml, either alone or in combination, reduced proliferation of the cells significantly.
Conclusion: The data presented in our study provide evidence for direct action of anti-inflammatory cytokines on oligodendrocyte precursor cells survival.
Keywords: Oligodendrocyte Precurser Cells, LPS, IFN Gamma

Ps-80: Glucocorticoid and Estradiol Treatments Reprogram Bone Marrow Stem Cell Maturation

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Objective: A novel mechanism for the cardioprotection may include bio-signal combination actions. There are gender differences in anti-inflammatory properties and bone marrow stem cells (BMSC) ability to heal injured tissues. Glucocorticoids are suggested to suppress induced apoptosis *in vitro* by reducing cell death signals.
Materials and Methods: Herein, we aimed investigating growth and differentiation properties of patient-isolated BMSCs. Cultured MNCs were treated with small lipophilic compounds for 72 hours and 7 days, respectively. Beside differentiation capacities, BMSC morphology, proliferation, the induced expression of surface markers was assayed.
Results: The estrogen was found to induce cell proliferation and significantly the expression of CD133 marker. In synergism, compounds induced both, the expression of CD133 and CD71 markers, while increasing myogenic and decreasing osteogenic capacity was observed. The estrogen function was more prevalence with LPS/TLR2 stimulation.
Conclusion: The bio-signals are able to delay BMSCs maturation by reprogramming gene expression, while provoking the cells toward distinct differentiation.
Keywords: Cardioprotection, Reprogramming, Bone Marrow Stem Cells, Growth Capacity

Ps-81: Effect of Chitosan on Osteogenic Properties of Mesenchymal Stem Cell of Exfoliated Deciduous Teeth

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Objective: The exfoliated human deciduous tooth contain multipotent stem cells [stem cell from human exfoliated deciduous tooth (SHED)] that identified to be a population of highly proliferative and clonogenic. These cells are capable of differentiating into a variety of cell types including osteoblast/osteocyte, adiopcyte, chondrocyte and neural cell.The aim of this study was to evaluate the differentiation of SHED to osteoblast in standard osteogenic medium and comparing the results with medium which supplemented with glucosamine in form of chitosan.
Materials and Methods: Dental pulp cells were isolated from freshly extracted primary teeth, digested with 4 mg/ml collogenase/dispase, and grown in Dulbecco's modified Eagle's medium with 10 percent fetal bovine serum. The clonogenic potential of cells was performed after 3 weeks of culture. Flowcytometric analysis, performed at day 21 of culture to identify surface markers of mesenchymal stem cells. The cells from 3rd passage use for osteogenic differentiation in routine osteoinductive medium. Chitosan (10 µg/ml) was added to the

culture medium of case group. Alizarin Red Staining and Alkaline Phosphatase (ALP) activity were done to evaluate osteogenic differentiation in the developing adherent layer on the third passage.
Results: The colonogenic efficiency was more than 80%. Flowcytometric analysis showed that expression of mesenchymal stem cell marker CD90, CD105 and CD146 were positive in SHED, while hematopoietic cell marker CD34, CD45 and endothelial cell marker CD31 were negative. . Quantitative analysis of Alizarin Red Staining demonstrated that: mineralized nodule formation was higher in the group supplemented with glucosamine (chitosan). Results from Alkaline Phosphatase activity test, on day 21, demonstrated higher ALP activity in group supplemented chitosan.
Conclusion: Stem cells isolated and cultured from exfoliated deciduous teeth pulp, can differentiated to osteoblast. Addition of chitosan could be beneficial to promote osteogenic differentiation of these cells.
Keywords: Stem Cell, Deciduous Tooth, Glucosamine, Chitosan

Ps-82: Pericardium Membrane: Well Suited for Myocardial Tissue Engineering

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Objective: Cardiovascular disease hold the highest mortality rate among other diseases and this fact reveals current limitations of common therapies. In recent decade, role of 3-dimentional scaffolds has been highlighted by their principal contribution in treatment of heart failure postmyocardial infarction. Scaffolds of natural origin which resemble more to the damaged tissue of interest, seems to be more suitable for replacement of necrotic cardiomyocytes and restore of the damaged extracellular matrix. In this study we investigated human pericardium membrane as an appropriate scaffold for myocardial tissue engineering.
Materials and Methods: Human pericardium (HP) was treated with standard decellularization solution consist of hypotonic lysis buffers. Tissues were rinsed in DPBS under gentle agitation at 4°C, and then were evaluated by light microscopy. Then acellular HP (AHP) was lyophilized up to 24 hours. followed by evaluation of its mechanical and swelling properties. In order to prepare the AHP for cell seeding, thick fibrous Layer of, about 250 µm, was removed from it by cryostat. Cardiac progenitor cells were isolated from heart atrium biopsy. Sca-1+ cardiac progenitor cells were sorted by FACS technique and then seeded on sterilized AHP. After 7 days in culture, the membranes were analysed for proliferation assay, SEM and histological staining.
Results: Our results indicated that the number and size of porosities in AHP significantly increased after lyophilisation. The swelling ratio of AHP after 24 h was approximately 83% . MTS assay showed that cells have a growth rate similar to control group on TCPS in 3, 7 and 14 days after cell seeding. Histological and SEM microscopic results showed that cells attached on the surface of membrane and connected into ECM. With Masson's trichrome staining collagen fibres were observed among cells and exibited normal morphology.
Conclusion: Our data showed that natural pericardium membrane as a suitable scaffold could be used in cardiac tissue engineering.
Keywords: Myocardial Tissue Engineering, Pericardium Membrane, Sca-1+ Cardiac Progenitor Cells

Ps-83: Investigation of Simvastatin Effects on Differentiation of Periodontal Ligament cells (PDLs) to Cementoblasts

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Objective: Simvastatin is known as a treatment for hypercholesterolemia, although recently it is shown that this drug is able to stimulate bone formation. The goal of this study was to show whether similar phenomenon can occur in dental tissues and simvastatin can stimulate the differentiation of cementoblasts from Periodontal Ligament (PDL) cells.
Materials and Methods: Human PDL cells were cultured and treated with different concentrations of simvastatin (10-6, 10-7, 10-8 and 10-9 µg /µl). Then for cementobastic differentiation, expression of Bone Morphogenetic Protein-2 (BMP-2) and GLUT-1 was assessed using quantitative real time polymerase chain reaction (qRT-PCR) after 24, 48 and 72 post culture.
Results: GLUT-1 and BMP-2 expression were significantly increased in cultures containing 10-6, 10-7, 10-8 and 10-9 µg /µl of simvastatin with its major effect in the concentration of 10-7 µg /µl and after 72 hours of culture.
Conclusion: Optimum concentrations of simvastatin can accelerate the differentiation of PDL cells to cementoblasts and has the potential to be used in periodontal regeneration.
Keywords: PDL, Simvastatin, BMP-2, GLUT-1

Ps-84: Expression of HLA-G5 in Adipose Derived Stem Cells (ASCs) of Breast Cancer Patients Compared to a Normal Group

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Objective: Adipose derived stem cells (ASCs) are important for their presence in tumor microenvironment due to their immunomodulatory effects on both innate and adaptive immune responses. ASC-mediated immunesuppression mainly acts through the secretion of soluble molecules such as IDO, NO, PGE2 and HLA-G5. The aim of this study was to assess the expression of HLA-G5 in adipose derived stem cells isolated from breast cancer patients compared to those isolated from normal individuals.
Materials and Methods: ASCs were isolated from 15 breast cancer patients and 10 normal adipose tissues using collagenase digestion. HLA-G5 was assessed in them by q-RT- PCR method using appropriate primers.
Results: There was no significant difference in the expression of HLA-G5 in ASCs of breast cancer patients in comparison with those isolated from normal individuals.
Conclusion: Although recent studies showed ASC mediated immunomodulatory effect of HLA-G5 in cancer, expression of this mediator is not different between breast cancer and normal individuals.
Keywords: Adipose Derived Stem Cell (ASC), HLA-G5, Breast Cancer

Ps-85: Cytotoxicity Comparison of MTA Fillapex Sealer, AH26 Sealer and Angelus MTA Materials on PDL Fibroblast Cells. An *In Vitro* Study

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Objective: An ideal root filling materials must have the ability to seal the root canal system in three dimensions, be nontoxic and well tolerated by periradicular tissues, have dimensional stability, nonabsorbability, and not affected by the presence of moisture. MTA has good biological properties, and it is a mineralization-inducing materials with different applications in endodontics. But owing to its physical properties, this materials is not recommended as a root canal sealer. However, a resin sealer based on mineral trioxide aggregate (MTA Fillapex) was recently produced with this indication. Because sealers are in contact with the periodontal tissues, it is important to know their cytotoxic effects. So the purpose of this study was to evaluate the cytotoxicity of MTA canal sealer (MTA Fillapex) compared with Angelus MTA cement and AH26
Materials and Methods: Human periodontal ligament fibroblast cells (HPLF) were cultured in media previously exposed to MTA Fillapex, Angelus MTA cement and AH26 for 24 hours to 1week. Cytotoxicity was evaluated by methol-thiazol-diphenyl tetrazolium (MTT) assay in spectrophotometer to check cell survival and viability rate.
Results: Cell viability remained above 50% in Angelus MTA group after 24 hours and declined after 48 hours and1week. AH26 induced an intermediate cytotoxicity in 24 hours but the cytotoxicity remained unchanged after 48 hours and 1week. MTA Fillapex was less toxic than AH26 after 24 hours but the toxicity of both materials was the same at 48 hours and 1week.
Conclusion: This study showed that angelus MTA is less toxic in comparison with MTA Fillapex sealler and AH26.
Keywords: PDL Fibroblast Cells, Cytotoxicity, MTA Fillapex, Sealer, MTA

Ps-86: Myricetin: As A Therapeutic Intervention for Meningioma

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Objective: Brain tumors are important tumors with high lethal rate and devastating impact on life quality of patients. Meningiomas are various sets of brain tumors arising from the meninges. Urgent action for novel therapies has contributed to great emphasis on the identification of cancer cells in these tumors. Myricetin has been recently introduced with anti-proliferative potent effects in a variety of tumors. Aim: Here, we aimed to see the effects of Myricetin on the proliferation rate and viability of meningioma tumor cells.
Materials and Methods: Brain tumor tissues obtained from patients diagnosed with meningioma were transferred to lab under sterile condition, washed with PBS, cut into small pieces, digested with Collagenase type 1 and cultured in the tissue culture falsk containing DMEM and 10% FBS. As cells arise to passage 3, they were treated with different concentrations of Myricetin (0, 0.01, 1/250, 1/500 and 0.001 M) for 24, 48 and 72 hours and then IC 50 was evaluated with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT).
Results: Results showed that, meningioma tumor cells have plastic adherent ability. IC50 for treated cells was at concentration of 0.001 M after 48 hours post cul-

ture.
Conclusion: Our study showed that Myricetin has proper influences on meningioma cancer cells eradication. However, further investigation is needed to confirm its anticancer potentials and it may consider as a therapeutic intervention for meningioma in future.
Keywords: Meningioma, Cancer Cells, Myricetin

Ps-87: Expression of Tumor Promoting Mediators in Adipose Derived Stem Cells (ASCs) after Transfecting with SDF-1 and IP-10 Plasmids

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Objective: Mesenchymal stem cells (MSCs) are recently introduced as novel immunological gene carriers for treatment of cancer. The balance between the expression of angiogenic and anti-angiogenic factors such as IP-10 and SDF-1 regulates the neovascularization within the tumor. Here, we compared the expression of important tumor promoting mediators in IP-10-transfected ASCs compared to those transfected with SDF-1.
Materials and Methods: ASCs were isolated from adipose tissue of normal subjects undergoing cosmetic mamoplasty using collagenase. ASCs were transfected with IP-10 or SDF-1 propagated plasmids by electroporation method and Lipofectamin 2000. Expression of SDF-1, CXCR4, IP-10, Bcl-2, MMP2, IL-10, IGF-1 and VEGF was detected in transfected ASCs using quantitative real-time polymerase chain reaction (qRT-PCR).
Results: Expression of SDF-1, CXCR4, Bcl-2, MMP2, IL-10, IGF-1 and VEGF was upregulated in SDF-1-transfected ASCs. VEGF showed an eight-fold lower expression in ASCs transfected by IP-10.
Conclusion: Anti-angiogenic chemokines may modulate the tumor promoting properties of ASCs and introduced as novel candidates for tumor immunotherapy but further studies are certainly required.
Keywords: Adipose Derived Stem Cells, IP-10, SDF-1

Ps-88: Screening of Transfected Stem Cells with Replicable Minivector and its Conversion to Minicircle DNA

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Objective: Episomal minicircle DNAs, devoid bacterial backbone exerts ideal vehicles for cell and tissue transfection. New generation of these vehicles carrying Scaffold/matrix attachment region (S/MAR) element causes long-term expression of transgene without selection. The sole concern is remaining of un- transfected cells among transfected dividing cells population. Here, we developed a simple *in vitro* method to generate supercoiled, transfection-ready minicircles in a semi-synthetic method in purpose of long term episomal transfection of target genes in stem cell lines. First, minivector including eukaryotic antibiotic resistance gene was constructed for primary selection. Then it was converted to minicircle DNA by means of Cre recombinase.
Materials and Methods: At the first step a DNA fragment encoding EGFP, S/MAR elements and SV40 promoter was amplified using pGL268 pEpi-FGM18F plasmid. Then, puromycin resistance and its promoter sequences were amplified introducing LoxP at both ends. Each amplified fragments were treated with different restriction enzymes and ligated to produce a minivector. This construct was transfected into stem cells and stable cell line achieved after two days of treatment by puromycin. The next transfection with Cre recombinase expression vector was accomplished to extract antibiotic resistance gene and convert minivector to minicircle DNA.
Results: Minicircle DNA carrying EGFP-S/MAR were constructed successfully by semi-synthetic method. Generated minicircle retained functionality to produce EGFP in several passages and generations.
Conclusion: Here we reported construction of an efficient minivector containing S/MAR elements and an antibiotic screening step for isolation of transfected cells. Moreover here we modified a new and efficient system for creation of purified minicircles avoiding bacterial hosts to propagate them.
Keywords: Episomal, Minicircle DNA, S/MAR Element, Stem Cell

Ps-89: Adipose Tissue-Derived Mesenchymal Stem Cells Differentiation into Neural Retinal Cells through PAX6 Gene Over-Expression

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Objective: Adipose- derived mesenchymal stem cells (ADSCs) are a kind of mesenchymal stem cells which have revealed many advantages over other stem cell sources. Pax6 gene is highly conserved among species and plays an important role in eye morphogenesis. Here we studied the ADSCs differentiation into neural retina cells as a tool for cell therapy in retinal degenerative disorders.
Materials and Methods: The coding region of human Pax6 (+5a) gene isoform was synthesized and cloned into pUC57 cloning vector. The coding sequence of Pax6 (+5a) was then digested by BamHI and XhoI restriction enzymes and subsequently subcloned into pLEX- IRES- Pur Lentiviral vector. The recombinant construct was confirmed by PCR, digestion and finally sequencing. For isolation of ADSCs, human adipose tissue was obtained from abdominal subcutaneous tissues of patients undergoing abdominoplasty. Isolated ADSCs were characterized by their differentiation potential into adipocytes and osteocytes, moreover their surface markers including CD34, CD45, CD73, CD 90 and CD105 were examined by flowcytometery. The lentiviral particles were produced in HEK293T cells and subsequently the ADSCs were transducted. The successful transducted cells were selected by puromycin resistance.
Results: We constructed a vector expressing Pax6 protein. The isolated ADSCs were successfully differentiated to adipocytes and osteocytes which stained with Oil Red-O and Alizarin Red staining respectively. Flowcytometric analysis of surface markers indicates the high purity (99.8%) of isolated ADSCs. Proliferation capacity of differentiated cells was decreased strongly and cell death was observed. After 5 days culture in the presence of puromycin, cells developed a neuron-like configuration. Pax6 and Nestin expression were confirmed by immunocytochemistry(ICC). ICC for other retinal cell markers and Real-time PCR tests are undergoing.
Conclusion: The ADSCs were differentiated to some cell types of neural retina. Although more researches are under investigation.
Keywords: Adipose Tissue-Derived Mesenchymal Stem Cells, Human Pax6 Gene, Neural Retina

Ps-90: The CD44/24 Populations Isolated from A549 Cell Line Exhibit Similar Capacity *In Vitro* Studies

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Objective: Lung cancer is the leading cause of cancer-related death worldwide. Cancer stem cells (CSC) are a rare population of undifferentiated tumorigenic cells responsible for tumor initiation, maintenance and spreading. We aimed to identify stem-like populations in A549, as human lung adenocarcinoma epithelial cell line, and to investigate CSC features including expression of putative surface markers, clonogenic and sphere forming capacity of these populations.
Materials and Methods: Non-sorted A549 cells were studied for clonogenic and sphere forming capacity. Flow cytometric profiles for CSC markers aldehyde dehydrogenase 1(ALDH1), CD24, CD29, CD44, CD49, CD133 and ABCG-2 were determined in A549 cell line. Cells were sorted and assessed for clonogenic and sphere forming capacity.
Results: In non-sorted A549 cells, clonogenic and sphere forming capacity were 49.58% and 0.01% respectively. Expression of ALDH1Variant activity exhibited a range from 0.5% to 21.3%. CD44+ (69.54%-97.6%) and CD24+ (55.91%-86%) were highly abundant, on the other hand expression of ABCG-2 and CD133 markers were 0.93% . In A549 cells, expression of CD29 marker was rare. Double positive CD29/44 and CD44/133 populations were rare, but double positive CD44/24 population exhibited a range from 18.60% to 79.4% in A549 cell line. Both double negative and positively CD44/24 and CD44+/24- sorted populations displayed similar clonogenic and spheres forming capacity.
Conclusion: In this study, a panel of candidate CSC markers in A549 human lung adenocarcinoma epithelial cell line were assayed. Expression of ABCG-2, CD133 and CD29/44 and CD44/133 markers were very low. Consequently it was not possible to sort the cells using these markers. *in vitro* studies of both double negative and positively CD44/24 and CD44+/24- sorted populations suggested that CD44 and CD24 are not suitable markers for A549 lung cancer cell line, because these sorted CD44/24 populations showed similar clonogenic and sphere forming capacity. However this experiment needs to be complemented by and *in vivo* study for tumorogenicity of these populations in NOD-SCID mice.
Keywords: Lung Cancer, A549 Cell Line, Cancer Stem Cells, CD44/24, Cell Sorting

Ps-91: The Effect of Hydrostatic Pressure on Growth Factor Induced Chondroinduction of Human Adipose-Derived Mesenchymal Stem Cells

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Objective: Tissue engineering using stem cells is a promising method for cartilage regeneration. In articular cartilage, chondrocytes sense mechanical signals such as hydrostatic pressure; and regulate the metabolism of proteoglycans and collagens to maintain homeostasis of extracellular matrix. Mimicking these conditions may improve chondroinduction of stem cells *in vitro* and can be used in cartilage tissue engineering applications.
The aim of this work is to study the effect of intermittent hydrostatic pressure on growth factor induced-chondroinduction of human adipose-derived mesenchymal stem cells (hASCs).
Materials and Methods: After isolation of hASCs from abdominal fat tissue, characterization by flow cytometry and assessing multilineage differentiation potential, pellets of passage 3 cells were used in experimental groups including: control group: pellets in routine culture medium, chemical group: pellets in chondrogenic differentiation medium containing TGF-β1, dexamethasone and ascorbate-2-phosphate for 10 days, and chemical-mechanical group: pellets in chondrogenic medium subjected to 3 MPa cyclic hydrostatic pressure (0.5 Hz) for 7 consecutive days form 4th day of pellet culture (4 hours/day) using a hydrostatic pressure device previously designed and fabricated in National Cell Bank of Iran. Real-time PCR method was used to examine the expression of aggrecan, as an important chondrogenic marker.
Results: Cell surface protein expression examined by flow cytometry was highly characteristic of MSCs. Multilineage differentiation confirmed the multipotency of isolated hASCs. According to Real-time PCR results, application of cyclic hydrostatic pressure in chemical-mechanical group resulted in a significant increase in aggrecan expression (by 11 folds) compared to the chemical group.
Conclusion: Higher expression of aggrecan, as an important chondrogenic gene, in the chemical-mechanical group, shows that mechanical signals are important factors in chondrogenesis and hydrostatic pressure can be used as an effective tool in hASCs chondrogenic differentiation and cartilage tissue engineering.
Keywords: Mesenchymal Stem Cells, Hydrostatic Pressure, Chondrogenic Differentiation

Ps-92: Differential Expression Pattern and Protein Level of PPARγ during Retinoic Acid Induced-Neural Differentiation of Human Embryonic Stem Cells

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Objective: PPARγ is considered as a ligand induced-nuclear receptor and a transcription factor in many of cell types especially adipocytes. Previous studies have indicated an increment in PPARγ1 expression level during neural differentiation of mouse embryonic stem cells. In addition, bioinformatics data have considered four isoforms for human PPARγ which we observed high level expression of PPARγ1and3 during neural differentiation of human embryonic stem cells (hESCs) compare to less expression of PPARγ4 and absence of PPARγ2. To pursue our studies, this study was designed in aim of assessment the expression level of total mRNA PPARγ during neural upon retinoic acid (RA) treatment compare to absence of RA induction.
Materials and Methods: Treated human embryonic stem cells with noggin and bFGF were induced to differentiate into neuroectodermal cells with retinoic acid treatment for 6 days. Subsequently, cells were grown without retinoic acid for more 6 days to promote rosette formation. Neural tube-like structures were emerged after exposure cells with bFGF for extra 6 days. Finally neural tube-like structures were isolated and plated on laminin and polyL-ornithine coated dish for up to 12 days until neural formation completed. Moreover, neural differentiation was performed using noggin induction in the absence of RA state. RNA and protein were extracted from hESCs, and neural cells in all stages of experiments, which were applied for real time PCR and western blotting respectively.
Results: Our results indicated that despite an increase mRNA level of PPARγ upon RA treatment, no significant difference in PPARγ protein content were observed during neural differentiation. Interestingly, there was no significant increase in mRNA level of PPARγ during neural differentiation when noggin was applied for induction of this process. Taken together, although PPARγ mRNA showed an elevated expression level during neural precursor cells (NPCs) formation compare to hESCs and neural cells, this increase was not observed in protein.
Conclusion: It can be concluded that this difference between mRNA and protein of PPARγ may be a reflection of regulatory mechanisms by presumable PPARγ-specific miRNA or by the ubiquitin-proteasome degradation system. However further experiments are needed to clarify this phenomenon.
Keywords: PPARγ, mRNA, Protein, hESC, Neural Differentiation, Retinoic Acid

Ps-93: Transplantation of Human Induced Pluripotent Stem Cell-Derived Neural Progenitors to Rat Crushed Optic Nerve

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Objective: We examined the survival, integration, differentiation and functional repair potency of human induced pluripotent stem cells- derived neural progenitors (hiPS-NPs) following transplantation into the retina of rats with crushed optic nerve.
Materials and Methods: The Royan hiPs were induced to NPs using Noggin and Retinoic acid during 3 week. Q RT- PCR, Immunocytochemistry and flowcytometry were used to detect markers genes and proteins of neural progenitor cells. The expression of ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (bFGF) and Insulin-like growth factor 1(IGF1) by hiPS-NPs were detected by ElISA. One hundred rats were randomly divided into four groups: normal control, optic nerve crushed rats injected with PBS, optic nerve crushed rats injected with hiPS-NPs, or injected with dead hiPS-NPs. Cells was labeled by red fluorescent CM-DiI and injected into the vitreous cavity one day after breaking inner limiting membrane by acid aminoadipic acid. Visual evoked potential recording was done for evaluating functional recovery of optic apparatus. Transplanted cells fate was then evaluated using Immunohistofluorescence.
Results: hiPS-NPs characterization showed anterior cell characteristics with high expression level of PAX6. ELISA showed neurotrophic factors secretion by hiPS-NPs. Functional analysis using VEPs showed significant amplitude recovery in animals transplanted with live cells. Sixty days after transplantation hiPS-NPs were integrated into the ganglion cell layer of retina and some of them expressed markers of ganglion cells. Retrograde labeling with DiI showed higher number of projection cells in retina.
Conclusion: hiPS-NPs transplantation was able to protect retinal ganglion cells after optic nerve injury, and integrated and differentiated in to ganglion cell layer. These cells may provide new therapeutic approach for traumatic optic nerve diseases.
Keywords: Human Induced Pluripotent Stem Cell, Neural Progenitors, Optic Nerve Crush, Visual Evoked Potential, Transplantation, Rat

Ps-94: Epigenetic Analysis of Human Embryonal Stem Cells during Neural Differentiation

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Objective: Differentiation of embryonal cells from a pluripotent to a committed state involves global changes in genome expression patterns, critically determined by chromatin structure and interactions of chromatin binding proteins. The dynamics of chromatin structure are tightly regulated by multiple epigenetic mechanisms such as histone modifications and histone variants incorporation.
Materials and Methods: In the current work, a human embryonal carcinoma stem cell line (NT2/NTera2) was induced to neural differentiation by retinoic acid (RA)-treatment, and electrophoretic pattern of histone extracts were compared in different stages, using SDS-PAGE as well as Acid-Urea gel electrophoresis. The observed changes were further analyzed by mass spectrometry and confirmed using western blot technique. Using chromatin immunoprecipitation (ChIP) coupled with real-time PCR, it was also checked the genome incorporation of the epigenetic marks on the regulatory regions of several marker genes involved in stemness and differentiation, respectively.
Results: In addition to dramatic morphological changes, a significant variation was observed in histone marks through different stages of neural induction, under different culture conditions. Some histone variants such as H1.x and H2A.Z showed dynamic expression levels in this process, parallel to a global change in acetylation/methylation pattern of histone H3, all of them known as key indicators of chromatin changes from stemness to differentiated state.
Conclusion: These results showed the dynamic interplay of histone modifications/variations in regulating gene expression pattern of stem cell fate and function.
Keywords: Epigenetic, Histone Modification, Variation, Neural Differentiation, Retinoic Acid

Ps-95: Modulation of CXCR4 Expression on Cord Blood Stem Cells

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Objective: CXCR4 is critical adhesion molecule in homing process. Modulation of its expression on cord blood (CB) CD34+ could overcome delay following cord blood transplantation. Complicated network of growth factors including cytokines and neuropeptides in microenvironment has important role in regulation of

this adhesion molecule. So, we aimed to assess the role of two neuropeptides Substance P (SP) and Calcitonin gene related peptide (CGRP) in addition to cytokine cocktail on CXCR4 expression.
Materials and Methods: CD34+cells purified from CB were cultured in a serum-free liquid culture system. Different concentrations of SP and/or CGRP were used in combination with cytokine cocktail. Protein and genomic levels of CXCR4 was assessed by flowcytometry and real time RT-PCR.
Results: Our data show increased CXCR4+CD34+ cells cultured with SP and/or CGRP by day 7. Concentration 10-9M either SP or CGRP increased the genomic expression of CXCR4 molecule by day 11 compare to control group.
Conclusion: Our experiment indicates that SP and CGRP induce CXCR4 protein expression in 7 days culture and enhance its genomic expression. Consequently, over expression of CXCR4 improves engraftment of CB CD34+ cells.
Keywords: CXCR4, Cord Blood, Stem Cell

Ps-96: The Effect of Substance P on Very Late Antigen -5 (VLA-5) Expression in Cord Blood CD34 + Cells

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Objective: To facilitate the homing process of cord blood (CB) stem cell, the overexpression of cell adhesion molecules -esp. those involved in migration process- could be helpful. Factors such as microenvironment and released mediators regulate this process. Additionally, according to existing documents concerning the presence of substance P (SP) receptor on CB CD34+cells, we aimed to explore effects of SP on very late antigen (VLA)- 5 expression on CB CD34+ cells.
Materials and Methods: CD34+cells derived from CB, were cultured in Stemspan culture and were treated with various concentrations of SP in combination with cytokine cocktail of SCF, FL, TPO, IL3 and IL6. Control groups were treated with cytokine cocktail. VLA5 expression was checked by flowcytometry.
Results: Our results show significant raise in percentage of SP treated cells at 10-11 M following 7 days cultivation as compared to control group. Additionally median flowcytometric intensity of VLA5 at corresponding culture period and SP concentrations were significantly increased compare to freshly purified CD34+cells at day 0.
Conclusion: Our data suggest that using SP neuropeptide help to maintain or increased VLA5 on CB stem cells and this would be beneficial in homing process of cells during transplantation.
Keywords: SP, VLA-5, Cord Blood, Stem Cell

Ps-97: Is CD133 An Applicable Marker for The Isolation of Prostate Cancer Stem - Like Cells?

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Objective: A small population of cells in tumors which responsible for initiation, progression and tumor metastasis named cancer stem (like) cells. CD133 is a cell surface marker that has been used for isolation of normal and malignant stem cells. In the present study we determine the stem-cell-related functions of the CD133+ and CD133- prostate cancer cells.
Materials and Methods: LNCaP cells were cultured under standard conditions and were isolated up on CD133 expression using BD FACS Aria cell sorter. To characterized sorted cells, doubling time, colony assay and spheroid formation were performed on each group respectively. RT-PCR was performed to assess the expression levels of stemness genes (Oct4, Sox2, Nanog and Klf4).
Results: The expression of Oct4 and Nanog genes was not seen in LNCaP cells; however they expressed Sox2 and Klf4. There was no significant difference in doubling time, colony and spheroid formation between CD133+ and CD133- cells.
Conclusion: Our results suggested that the CD133+ is not specific marker for identification of prostate of cancer stem cells in LNCaP cell line, however in the cancerous tissue *in vivo*.
Keywords: Cancer Stem (like) Cells, CD133, Prostate Cancer

Ps-98: Human Endometrial Stem Cells Attain Oligodendrocyte Features in Vitro

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Objective: Human endometrial-derived stem cells (EnSCs) are the abundant and easy available source for cell replacement therapy. Oligodendrocytes are myelinating cells in the central nervous system (CNS) that form the myelin sheath of axons to support rapid nerve conduction. In CNS disorders, such as stroke, multiple sclerosis and spinal cord injury, demyelination of axons contributes to functional deficit.
Materials and Methods: The characterized cells were coaxed to oligodendrocyte differentiation by induction of bFGF, EGF and PDGF-AA (20 ng/ml) signaling molecules for 21 days and T3 (30 ng/ml) for 8 days. Differentiated cells were analyzed for expression of neuronal markers by RT-PCR and Immunocytochemistry.
Results: The flow cytometric analysis showed that EnSCs were positive for CD90, CD105, OCT4 and were negative for CD31, CD34. The result showed the expression profile of oligodendrocyte markers such as Nestin, Olig2, PDGFRa, CNP in the level of mRNA. The expression of Olig2, PDGFRa, A2B5 proteins in EnSCs were confirmed at 30 days post treatment (PT) by Immunocytochemistry.
Conclusion: The EnSCs can response to the signaling molecules which usually used for oligodendrocyte differentiation and can program to oligodendrocyte cells and may convince to consider these cells as a unique source for cell therapy of neurodegenerative disease.
Keywords: Endometrial Stem Cell, Oligodendrocyte, Differentiation, Neurodegenerative Disease

Ps-99: Endothelial Differentiation of Human Amniotic Epithelial Cells

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Objective: Amniotic epithelial cells (AECs) are closest layer of the placenta to the foetus. There are several reports that amniotic derived epithelial cells are pluripotent cells which can differentiate into different types of cells from three germ layers. However, there is no report on the differentiation of the AECs into endothelial lineage. Our goal was to evaluate the potential inducing effect of growth factors on differentiation of the AECs into endothelial cells.
Materials and Methods: The amniotic membrain (AM) was detached from human placenta and was digested by trypsin-EDTA for the AECs to be extracted. The extracted AECs were characterized, based on their expression of pan-cytokerain, assessd by immunocytochemistry. They were cultured in gelatin coated plates, in DMEM/F12 medium contained 10% FBS, 1% penicillin/streptomycin, and growth factors including EGF, VEGF, bFGF and BMP-4. The expressions of endothelial markers were evaluated by immunocytochemisty.
Results: The extracted AECs had more than 90% viability confirmed by Trypan Blue and MTT assay. Expression of pan-cytokeratin confirmed that isolated cells were AECs. Endothelial differentiation was induced within 12 days in the AECs and their morphology was changed after addition of growth factors. 20% expression of Von Willebrand factor (vWF) was considered the minimum acceptable expression achieved by 25 ng/ml of VEGF. The percentage of this marker was increased by induction of BMP-4 signaling.
Conclusion: The AECs are embryonic stem cell-like cells that have pluripotent properties. Since the AECs do not express HLA-A, B or DR, the *in vivo* immunologic rejection of them has not been reported. Based on these properties and also differentiation characteristic of the AECs to endothelial lineage, they are suitable cell source for future pre-clinical and clinical studies on vascular diseases.
Keywords: Amnion, Epithelial Cells, Endothelial Cells, Differentiation, BMP-4

Ps-100: Progression of *In Vitro* Meiosis and Development of Ovarian Germ Cells in Chicken Embryos

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Objective: Understanding the mechanisms that lead to the differentiation of spermatogonial and oogonial stem cells into sperm and oocyte through meiosis has been a fundamental quest for many years. In spite of recent progress in understanding the onset of meiosis in mammalian germ cells, our knowledge on the mechanisms involved in meiosis of chicken germ cells is much more limited. The first step to study the mechanisms involved in meiosis is to culture and development maintain the gonads *in vitro*, where factors which might influence the onset of meiosis could be studied in isolation. In this study, the ovaries of the stage 36 chicken embryo were explanted under various conditions, and progression of meiosis was evaluated after 6 days of *in vitro* culture by histological, immunohistochemical and gene expression methods.
Materials and Methods: Ovaries were dissected from embryos with or without mesonephous at day 10.5 of incubation (5 days before onset of meiosis) and were cultured in different media with or without the use of

agar slabs. After 6 days the ovaries were fixed, stained and subjected to immunohistochemical and RT-qPCR analyses.
Results: Our results show that the presence of mesonephros and agar slabs is critical for *in vitro* culture of chicken ovaries. Experiments using immunohistochemistry and gene expression analysis confirmed the development of meiosis.
Conclusion: In this study, we have developed an *in vitro* culture system and related histological and molecular analyses for chicken embryonic ovaries and germ cell development.
Keywords: *In Vitro*, Germ Cell, Chicken Embryo, Meiosis

Ps-101: Transplantation of Human Induced Pluripotent Stem Cell-Derived Oligodendrocyte Progenitors into Severe Injured Spinal Cord Rat

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Objective: Induced pluripotent stem cells (iPSCs) with the possibility of patient-specific cell lineage transplantation represent an attractive source for cell therapy in regenerative medicine. This study aimed to assess therapeutic potential of hiPSC-oligodendrocyte progenitors (hiPSC-OPs) in a severe injured spinal cord model in rats.
Materials and Methods: The hiPSCs-OPs derived cells expressing PDGFRα, NG2, A2B5, O4 specific markers were transplanted into severe injured spinal cord rats seven days following contusion injury. The severity of model was confirmed by histological assessments. The behavioral tests (plantar and BBB score) were examined for sensory response and locomotor function for 5 weeks after SCI.
Results: Behavioral tests (plantar and BBB) during 5 weeks following transplantation showed non-significant improvement in functional recovery and sensory responses.
Conclusion: Taken together, it seems that oligodendrocyte progenitors alone are not to be sufficient completely overcoming the biological complications after severe SCI.
Keywords: Cell Therapy, hiPSC-Ops, iPSCs

Ps-102: Isolation and Purification of Human Spermatogonial Stem Cells Using Different Cultural Systems

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Objective: The fate of spermatogonial stem cells, as the foundation of spermatogenesis are tightly regulated by some intrinsic factor and extrinsic factors from their nich. Along the biological understanding of these mechanisms, a major concern is improving the efficiency, evaluation of the safety of their clinical application. Because the mechanisms that involved in human spermatogenesis are complex and unknown, this study was designed to providing an appropriate *in vitro* system to proliferate and enrichment of hSSC.
Materials and Methods: The isolation of spermatogonial stem cells (SSCs) were performed using two step enzymatic digestions and plating method. The identity of the SSCs and sertoli cells was confirmed through immunocytochemistry. We were designed 4 various culture system: co-culture with patient own Sertoli cells, co-culture with normal sertoli cells obtained from a person with normal spermatogenesis and culture of SSC on un-coated dishes and culture of testis cells suspension. The number and diameter of colonies were evaluated during the 3 weeks of culture. The identity of the SSC and sertoli cells was confirmed by immunocytochemistry against PLZF, GFR-a1and vimentin. The expression profile of the several germ stem cell specific and pluripotency markers were assessed using quantitative RT-PCR.
Results: Significant differences were observed between the four groups ($p<0.05$), with higher mean in number and diameter of colonies for co-culture with testis cells suspension in compare with other groups ($p<0.05$). Analysis of marker expression revealed that there are higher expression of germ stem cell markers and lower expression of pluripotency markers in co-culture with Sertoli cells.
Conclusion: Our findings demonstrated that adult co-culturing of SSCs with Sertoli cells can influence spermatogonial proliferation *in vitro*.
Keywords: Human Spermatogonial Stem Cells, Colony Formation, Culture, Gene Expression

Ps-103: Could Co-Culture with Sertoli Cells Effect on Characteristics of Human Spermatogonial Stem Cells?

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Objective: Along the biological understanding of spermatogenesis, a major concern is improving the efficiency of the various cultural conditions and evaluation of the safety of the clinical application. Since the interaction between SSCs and their nich is capable of inducing self-renewal in SSCs, suggesting that this signaling pathways are promoted in culture of SSCs, by co-culture with normal sertoli obtained from healthy persons
Materials and Methods: Testicular cells isolated from human testis biopsies by two step enzymatic digestion and plating methods. The identity cells were confirmed through immunocytochemistry. We were designed various culture system: co-culture with patient own Sertoli cells, co-culture with normal sertoli cells obtained from a person with normal spermatogenesis and culture of SSC on un-coated dishes. Colonization was evaluated during the 3 weeks of culture. The expression of the α6 and β1 integrins and PLZF as germ stem cell specific markers and nanog, c-myc and oct-4 as pluripotency markers were assessed using quantitative RT-PCR on 2nd and 3rd weeks of culture.
Results: Our result showed higher mean in number and diameter of colonies in co-culture groups in the compare with control group. Our data was shown the higher expression of germ stem cell markers during the culture and lower expression of nanog in 3rd weeks in co-culture with patient own Sertoli cells. Co-culture with normal sertoli cells in revealed significant higher expression of PLZF. At 3rd weeks, there are significantly increased in expression profile of α6 and β1 integrins and PLZF in compare to our control group. Co-culture with normal sertoli showed higher expression of germ stem cell specific markers at 3rd weeks of culture versus co-culture of SSCs with the patient own sertoli.
Conclusion: Co-culture of human SSCs with sertoli cells has emerged as a suitable method for the enrichment of spermatogonial germ cells.
Keywords: Spermatogonial Stem Cells, Sertoli Cells, Culture, Human

Ps-104: Activation and Inhibition of WNT3A Signaling Pathway in Buffalo Embryonic Stem Cells; Effects of WNT3A, Bio and Dkk1

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Objective: The present study was aimed to study the transcriptional profile of the WNT3A signaling pathway and its effects on maintenance of pluripotency as well as on induction of differentiation in buffalo embryonic stem (ES) cells.
Materials and Methods: In this study the effects of Bio (0.5 μM), in combination with WNT3A (200 ng/ml), as an activator, and Dkk1(Dickkopf-1) (250 ng/ml), as an inhibitor, of WNT3A signaling pathway, on mean area of buffalo embryonic stem (ES) cell colonies and expression of β-CATENIN and pluripotency genes were studied. WNT3A transected ES cells were compared with MOC transfected colonies, either alone or in combination with Dkk1, for expression of β-CATENIN and the pluripotency genes. Data were analyzed by ANOVA, and statistical significance was accepted at $p < 0.05$.
Results: Real-time PCR analysis showed that, NANOG, β-CATENIN, OCT3/4 and C-MYC genes were down-regulated in Dkk1 treated group. Immunoblotting also revealed a dramatic decrease in the level of β-CATENIN in Dkk1 treated group while WNT3A treatment caused a slight increase. Supplementation of the ES cell medium with Bio, either alone or in combination with WNT3A, did not significantly ($p>0.05$) affect the mean area of buffalo ES cells. Expression of β-CATENIN and pluripotency genes was similar when Bio was added to different treatments. It was also observed that β-CATENIN and NANOG genes were up-regulated in WNT3A transfected colonies as compared to the control (MOC transfected colonies), while the β-CATENIN and pluripotency genes were down-regulated in the presence of Dkk1 in MOC transfected colonies as compared to other groups. Differentiation study showed that WNT3A results to formation of scaffold like structure and inhibition to neural cell differentiation when buffalo ES cells were cultured in stem cell differentiation media.
Conclusion: Intact WNT3A signaling pathway present in buffalo ES cells derived from in vitro fertilized, parthenogenetic and handmade cloned embryos. WNT3A resulted in formation of scaffold like structure in differentiated buffalo ES cells. WNT3A is compulsory for maintenance of pluripotency in buffalo ES cells.
Keywords: WNT3A, Buffalo, Embryonic Stem Cells, Pluripotency, Differentiation

Ps-105: Optimization of Buffalo Embryonic Stem Cell Culture System

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Objective: In order to retain an undifferentiated pluripotent state, embryonic stem (ES) cells depend on culture on feeder cell layers. However, use of feeder layers limits stem cell research design, since experi-

mental data may result from a combined ES cells and feeder cell response to various stimuli.
Materials and Methods: In this study first, the effects of FGF-2 (5 ng/ ml), LIF (1000 U) and Y-27632 (10 μM) in gelatin based culture were analyzed. After that feeder layer based culture and gelatin coat based culture, either alone or in the presence of feeder-conditioned media (CM) from fibroblast cells, were compared. Alkaline phosphatase and immunofluorescence staining (pluripotency and cell surface markers) were used to characterize buffalo ES cells. Data were analyzed by ANOVA, and statistical significance was accepted at $p < 0.05$.
Results: The results showed that Y-27632, in presence of FGF-2 and LIF, resulted to higher colony growth and increased expression of NANOG gene. Feeder-CM resulted in a significant increase in growth of buffalo ES cells on gelatin coated plates, however, feeder layer based culture showed better results than gelatin based culture. Feeder layer from buffalo fetal fibroblast cells support buffalo ES cells from in vitro fertilized, parthenogenetic and handmade cloned embryos for more than two years.
Conclusion: We developed a feeder free culture system that support buffalo ES cells for short term maintenance as well as feeder layer based culture that support long term maintenance of buffalo ES cells.
Keywords: Embryonic Stem Cell, Buffalo

Ps-106: Cell-Surface Glycosaminoglycans Inhibit Intranuclear Uptake but Promote Post-Nuclear Processes of Nano-Sized Polyamidoamine Dendrimer-pDNA Transfection

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Objective: Interaction of cell-surface glycosaminoglycans (GAGs) with nonviral vectors seems to be an important factor which modifies the intracellular destination of the gene complexes. Intracellular kinetics of polyamidoamine (PAMAM) dendrimer as a nonviral vector in intranuclear delivery of DNA, transcription and translation steps with regard to the cell-surface GAGs has not been investigated until now.
Materials and Methods: The physicochemical properties of the PAMAM-DNA complexes were characterized by photon correlation spectroscopy, atomic force microscopy, zeta measurement and agarose gel electrophoresis. The transfection efficiency and toxicity of the complexes at different nitrogen to phosphate (N: P) ratios were determined using various *in vitro* cell models (human embryonic kidney cells, chinese hamster ovary cells and its mutants lacking cell-surface GAGs). Intranuclear uptake, transcription and translation expression of the particles were determined using optimized cell-nuclei isolation with quantitative real-time PCR and luciferase assay.
Results: Physicochemical studies showed that PAMAM G5-DNA binds DNA efficiently, forms small complexes with high positive zeta potential and transfects cells properly at N:P ratios around 5 and higher. The cytotoxicity could be a problem at N: Ps higher than 10. GAGs elimination caused nearly one order of magnitude higher DNA nuclear uptake and more than 2.6-fold higher transgene expression than CHO wild-type cells. However, neither AUC of nuclear uptake, nor AUC of transgene expression affected significantly by only cell-surface heparin sulphate proteoglycans (HSPGs) elimination and interesting data related to the effect of GAGs on intranuclear pDNA using PAMAM as delivery vector have been reported in this study.
Conclusion: Presented data shows that the rate-limiting step of PAMAM-DNA particle transfection is located after delivery to the cell nucleus and GAGs are regarded as an inhibitor of the intranuclear delivery step, while slightly promotes transcription or translation process.
Keywords: Glycosaminoglycan, Polyamidoamine Dendrimer, Transfection, Cell-nuclei Isolation, Intracellular Kinetics

Ps-107: Comparing Aligned and Random Nanofibrous PCL Scaffolds on Neural Differentiation of Mouse Embryonic Stem Cell

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Objective: Treatment of spinal cord injury is very complicated and create many challenges for scientists. Aligned and random nanofibrouse poly caprolactone (PCL) scaffolds which were produced by electrospinning teqnique can mimic from the extra cellular matrix (ECM) and be good candidate for nerve tissue engineering. Embryonic stem cells are the one of the choice for cell therapy in tissue engineering because of their easily cultured and capability of differentiation to all

lineage. In this study, the differentiation of mouse embryonic stem cells to neural leanage in aligned and random nanofibrous PCL scaffold was carried out and the effect of each, was studied.

Materials and Methods: Aligned and random pcl scaffolds were fabricated by electrospinning teqnique and their chemical and mechanical characterization were analyzed by scanning electron microscopy(SEM),tensile and contact angle test.The 3D embryoied bodies derived from mESCs were seeded in scaffolds and differentiated by inductive medium contained: F12,10%FBS,bFGF,RA,Ibmx,forskolin for 7 days. To observe neural differentiated markers, immonucytochemistry staining with polycolonal antibody of Map2 and β-Tub was carried out and neurite outgrowth was analyzed by SEM.

Results: Scanning electron microscopy images of aligned oriented nanofibers showed the extensive EBs with 2 polar orientation with neural trails parallel to fibers. EBs on random oriented nanofibers had multipolar direction. The results of immunocytochemistry staining showed the expression of two matured neural protein markers in the scaffold. Differentiated cells on aligned oriented scaffold had a long extensive appearance parallel to fibers of scaffolds,and on random oriented scaffold had shown irrigular expansion with shorter neural trails.

Conclusion: This study suggested that the aligned nanofiber scaffolds as a physical guidance, provides some topographical cues which is provided better substrate for neural differentiation of embryonic stem cells compared with random nanofiber scaffolds.

Keywords: Polycaprolactone (PCL), Scaffold, Neural Differentiation, Mouse Embryonic Stem Cell

Ps-108: CHIR99021 Mechanism of Action in Producing Germeline Pluripotent Stem Cell from Neonatal Mouse Testicular Cells

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Objective: According to previous studies, in culture of Spermatogonial stem cells some colonies, which morphologically resemble embryonic stem cells and express pluripotency markers, appear randomly. In this study, we produced some colonies which morphologically resemble embryonic stem cells using CHIR99021 and then characterized these colonies

Materials and Methods: We isolated testicular cells of neonatal mice according to the conventional tow steps method and cultured them in the presence of agonists of CHIR i.e., BIO and Kenpaulone and also in the presence XAV939 and PNU74654, β-catenin inhibitors. We then counted the number of colonies in each group and compared them with the positive control group (CHIR99021). Finally, we characterized the colonies at the level of mRNA for Oct4, Nanog, Vasa, and Gfrα1 by RT-PCR assay and at protein level for Oct4, Nanog, Gfrα1, and Plzf by Immunoflourescent assay. Furthermore, we investigated Apoptosis in the colonies, since the colonies became brownish after 8 days in culture. We investigated apoptosis and or necrosis both in situ and after enzymatic digestion using a cocktail of enzymes including Collagenaze type 4, Dispase, Hyalouronidase, Trypsin/EDTA and EGTA.

Results: There was a significant difference between the colony number in CHIR99021 group in comparison with CHIR+XAVE, CHIR+PNU, and CHIR+XAV+PNU groups which means CHIR99021 uses the canonical wnt pathway in producing these colonies. The colonies didn't express any of the studied genes either at mRNA level or at protein level. There was no sign of apoptosis in colonies in situ, but after enzymatic digestion nearly 80% of cells underwent necrosis

Conclusion: CHIR99021 uses canonical wnt pathway to produce the colonies in part and there may be other signaling pathways involved in producing these colonies. the true identity of the resultant colonies is not clear and furture studies will reveal this.

Keywords: Testis, Testicular Cells, Germline Pluripotent Stem Cells, CHIR99021, Mechanism of Action

Ps-109: Assessment of The Biological Activity of Prokaryotic Produced Recombinant Human IGFI on Neurosphere Formation of Mouse Embryonic Stem Cells

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Objective: Insulin-like growth factor-I (IGF-1), is a 7.6-kDa polypeptide with pleiotropic functions during development and growth in adults, plays an important role in proliferation and differentiation of various cell types especially in CNS. The main action of IGF-1 in CNS is exerted on proliferation and maturation of oligodendroglial cells and their precursors. Several studies have pointed neuroprotective effects of IGF-1 after CNS injury or disease. Therefore, IGF-1 may be applicable for treatment of neurodegenerative diseases. In

vitro studies have implicated IGF-1 in neuronal differentiation. As neural procurers cells (NPs) formation is a prerequisite matter for neural differentiation, we implemented the recombinant IGF-1 whether its activity increases NPs formation upon its supplementing to the media of mESCs. Thus, aim of this study was testifying recently produced recombinant IGF1.
Materials and Methods: mESCs were kept in an undifferentiated state in KDMEM supplemented with 15% ES-FCS. Neural precursor cells differentiation of mESCs was induced by culturing the cells in hanging drops to form embryoid bodies for two days. EBs were collected and underwent suspension culture in the presence of 1 mM retinoic acid for four days to form NPs. To investigate the effects of IGF1 on NPs formation, EBs in three groups treated with RA/recombinant produced IGF-1, RA/ Standard IGF-I and RA as control group. On day six, NPs formation and proliferation assay were performed by measuring the diameter of neurospheres and evaluating NPs specific marker.
Results: We showed that treating of EBs with recombinant IGF-1 and standard IGF-1 compare to untreated group, increased NPs formation and proliferation based on neurosphere diameter measurement. Evaluation of Nestin expression as specific NPs marker was performed too. Treating of neurospheres with RA and produced IGF-1 increased expression level Nestin compared to sole RA treatment.
Conclusion: Produced recombinant IGF-1incrased NPs formation and confirmed recombinant IGF-1 activity.
Keywords: IGF-1, Neural Differentiation, Neurosphere

Ps-110: Comparison Different Features and Differentiation Potency of Cells and Clumps of Bone Marrow and Adipose Tissue Derived Adult Mesenchymal Stem Cells

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Objective: Mesenchymal stem cells are multipotent stromal cells which show heterogeneity with self-renewal and multilineage differentiation potency. The aim of this study is analysis and comparing potential for clump formation of bone marrow and adipose tissue derived mesenchymal stem cells (BMSCs & AMSCs).
Materials and Methods: Bone marrow and adipose tissue derived stem cells are isolated and cultured. Morphology and viability of the cells are evaluated using hematoxilen-eosin and tripan blue staining method consequently. Clump formation of the cells is studied until passage 10 (P10). All clumps with < 2 mm diameter or with no stain are excluded. Both types of clumps are induced using osteogenesis and adipogenesis mediums for differentiate to osteocyte and adipocyte, consequently. Mediums are changed regularly every 3-4 days. Differentiation to adipose tissue are confirmed by the cellular accumulation of neutral lipid vacuoles are seen using oil-red staining and mineral production and deposition are seen using alizarin-red staining,about 3 weeks after serial induction.
Results: Growth rate, proliferation speed,and potency of clump formation was more in AMSCs ($p<0.05$). Both types of the cells had relatively the same morphology and were similar to spindle shape fibroblast but cells in clumps derive from bone marrow had more obvious spindle shape. Two types of clumps showed different morphology. AMSCs made more clumps. Degeneration speed in adipose derived clumps was more than other one. Differentiation rate in AMSCs was more than BMSCs.
Conclusion: Functions such as proliferation rate,viability, clump formation, and differentiation of stem cells are studied from two different anatomical sites. Results of this study showed that AMSCs are a more reliable source of seed cells with multiple merits such as minimal invasion, low morbidity, abundant yield, easy isolation, rapid expansion, good differentiation, and high potency in colony formation.
Keywords: BMSCs, AMSCs, Clump Formation, Differentiation Potency, Proliferation Rate

Ps-111: Spontaneously Differentiate of Mesenchymal Stem Cells into Lobulated Organization in Long Term Unchanged Culture

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Objective: Bone marrow-derived mesenchymal stem cells (MSCs) are multipotent cells with ability to differentiate into different types of cells and tissues. This differentiation might be occurred spontaneously in long time or induced using induction medium. In this study, we found that MSCs show certain spontaneous loubulation Organizations in culture in the absence of specialized induction reagents.
Materials and Methods: Mesenchymal stem cells were isolated from rat bone marrow based on their capacity to adhere to plastic culture surfaces. Confluent cells were split 1:2 using 0.25% trypsin and 0.02% EDTA, and passaged four times. The morphology and viability of MSCs were evaluated by Trypan Blue staining. Furthermore, MSCs were induced to osteocyte and adipocyte by corresponding osteogenic and adipogenic media for about three weeks and alizarine red S and oil red- O staining were done. Sub-confluent rat MSCs (passage 4) were cultured in the same medium for about four weeks. During this time the medium was neither changed nor supplemented by additional factors.
Results: This study showed that MSCs displayed adi-

pogenic and osteogenic phenotype after related inductions. In long term unchanged culture, MSCs displayed certain structural changes including variety of loubulation. loubulated structures represent that they are proceeding to more specialized tissues and shows their ability to spontaneous differentiation.
Conclusion: This study suggests that MSCs have the potential to produce different loboulation figures spontaneously in culture. Loubulation of stem cells might be used for aimed differentiation to some specialized tissues with loubulated frame such as breast, testis, thymus and so on.
Keywords: Mesenchymal Stem Cells, Loubulation Structure, Spontaneous Differentiation

Ps-112: MSCA1 A Good Cell Surface Marker for Bone Marrow Derived Mesenchymal Stem Cells but Not for other Sources

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Objective: Multipotent mesenchymal stromal cells (MSCs), previously known as "mesenchymal stem cells", are a group of multipotent and clonogenic cells. These cells were primarily found among the bone marrow (BM) stroma. A new marker called "mesenchymal stem cell antigen 1 (MSCA1)" has been found among BM-MSCs which is thought to be specifically expressed on MSCs. The Goal of this study was to assess presence of this newly found MSCs antigen on the mononuclear cells from other sources.
Materials and Methods: Five samples of BM and Granulocyte colony stimulating factor mobilized peripheral blood (G-CSF mobilized-PB) from the remnant part of bags used for bone marrow transplantation were collected. Mononuclear cells (MNCs) were isolated on the Ficoll layer and underwent flowcytometry for CD73, CD34, CD45, CD14, CD90, CD44, CD166, HLA-DR, CD271 and MSCA1.
Results: Among BM-MNCs 0.22 percent of cells were positive for MSCA1. Ninety percent of these cells were positive for CD271 and negative for CD45. The percent of MSCA1 positive cells in G-CSF mobilized-PB was raised up to 2.54 but 68% of these cells were positive for CD45
Conclusion: It has been shown that MSCs are a heterogeneous population and in some reports they do express CD271 and MSCA1 less than 60% also these two markers have been used widely for purification and enrichment of MSCs. Regarding our data MSCA1 in G-CSF mobilized-PB specifies cells from hematopoietic origin other than mesenchymal stem cells. MSCA1 is a good marker for isolation of MSCs from BM but not from other sources.
Keywords: Adult Stem Cells, Mesenchymal Stem Cell, Bone Marrow, Mesenchymal Stem Cell Antigen 1, Granulocyte Colony Stimulating Factor Mobilized Peripheral Blood

Ps-113: Culturing Limbal Stem Cells on Amniotic Membrane for Transplantation in Patients with Limbal Stem Cell Deficiency

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Objective: Limbal stem cells (LSC) have a prominent role in regeneration of cornea epithelial cells and have been applied in treatment of a vision disabling pathological condition known as limbal stem cell deficiency (LSCD). One the applied methodology is to culture LSCs followed by transplantation on patient's corneal surface. But this method has the non-deniable disadvantage of premature differentiation of stem cells. Amniotic membrane (AM) can support a convenient nurturing condition for LSCs, because of its adhesive and nutrimental properties. In the current study we focused on separation of LSCs and culturing them on AM in order to stop their premature differentiation before corneal transplantation.
Materials and Methods: Slices of cornea were obtained from eye bank and after being transferred to the lab, following procedure were applied to them: washing with PBS followed by immersing in Collagenase for 30 minutes and rewashing, cutting the digested tissue into very small pieces, incubation of slices for 48-72 hours in DMEM media containing 10% FBS and appropriate antibiotics, separating colony of cells by enzymatic digestion followed by transferring them onto AM surface.
Results: Cultured cells were morphologically confirmed to be undifferentiated limbal stem cells. After 3rd passage the cells were transferred on amniotic membrane surface. The cells formed colonies on amniotic membrane surface and also stayed undifferentiated.
Conclusion: Our results suggest for the achievement of transferring and culturing of LSCs onto the amniotic surface. This study is planned to be followed by

transplanting these cells on corneal surface and differentiating them into epithelial cells as an approach for treatment of LSCD. For further evaluations, we suggest to study the cell surface markers to confirm that the cultured cells are undifferentiated limbal stem cells.
Keywords: Limbal Stem Cell, Amniotic Membrane, LSCD, Corneal Cell Transplantation

Ps-114: Intravenous Administration of Mesenchymal Stem Cells Improve Functional Recovery after Traumatic Brain Injury in Rats

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Objective: Traumatic brain injury (TBI) is a major cause of mortaliy worldwide. The clinical studies have proven cell therapy as a major option to improve the brain function post trauma, and by possible regeneration of the nervous system.The aim was to investigate the role of intravenous administration of mesenchymal stem cells (MSCs) after experimental TBI in rats.
Materials and Methods: Rats were divided into two groups of TBI + PBS (control) and TBI + MSC (experimental). TBI was done based on model of Foda-Marmarou. MSCs were exposed with bromodeoxyuridine (Brdu) 48 hours before intravenous injection. The experimental group received 3×10^6 rat MSCs, labeled with Brdu, and PBS was injected to control group, into the lateral tail vein, 24 hours after TBI. The neurological severity score (NSS) was performed to evaluate the neurological function at 0, 1, 7 and 14 days after TBI. The rats were killed 14 days after TBI. MSCs migration and their differention to neurons and astrocyte cells were examined with immunohistochemistry technique.
Results: Results from NSS showed no significant differences between the groups of control and experimental at 1 and 7 days (3.5 ± 1.41 vs. 5.63 ± 2.44, $p=0.06$) and (2 ± 1.69 vs. 3.62 ± 1.99, $p=0.06$), respectively. However, motor deficits decreased significantly in the experimental rats when compared with control group at 14 days (0.75 ± 0.7 vs. 2.75 ± 1.83, $p=0.01$). Immunohistochemical studies showed that Brdu positive MSCs migrated via venous system to the cerebral tissue. Also, migrated MSCs to the injured brain were able to express neuronal (NeuN) and astrocytes (GFAP) markers.
Conclusion: Intravenous administration of MSCs seems to improve the functional recovery and neural cells regeneration after TBI in animal model. MSCs application may be suitable as therapeutic strategy for regeneration of CNS after TBI.
Keywords: Traumatic Brain Injury, Mesenchymal Stem Cell, Intravenous Administration

Ps-115: Sex Determining Region Y (SRY) Regulates Cancer-Marker Genes in NTera2 Embryonal Carcinoma Cell Line

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Objective: The SOX gene family which is characterized by the presence of a conserved DNA-binding high-mobility group (HMG) coding domain, encodes transcription factors that play critical roles in cell fate determination, differentiation and proliferation. Moreover,a number of SOX family members have been recently reported to be silenced in different types of cancer and proposed to act as a tumor suppressor in particular tissues. Sex determining region Y (SRY) is a member of SOX family proteins playing important roles in sex determination by initiating testis development from early bipotential gonads. Although previous studies extremely focused on the molecular mechanism of SRY in testis development,the possible role of SRY in cancer has not been investigated yet. In order to address this question, we looked into possible SRY-regulated genes and their levels of expression in a human embryonic teratocarcinoma cell line, NTera2, before and after onset of differentiation.
Materials and Methods: For this respect BCL2 and c-Myc were analyzed as cancer marker genes. Expression levels of c-Myc proto-oncogene and BCL2 at the mRNA level were determined by real time-PCR. Chromatin Immunopercipitation (ChIP) was performed using SRY antibody on chromatin extractions of NTera2 cells before and after differentiation, and SRY incorporation on the regulatory regions of the aforementioned marker genes were evaluated using real time-PCR.
Results: The results showed increased incorporation of SRY on the regulatory region of BCL2 and c-Myc after induction of differentiation, parallel with lower expression of these two cancer marker genes.
Conclusion: This finding suggests dynamic role of SRY as a transcription repressor for cancer-associated genes in cancer process.
Keywords: Embryonal Carcinoma Cell Line, SRY, SOX Gene Family, Transcription Repressor

Ps-116: The Effect of Mechanical Loading on Mesenchymal Stem Cell Targeted Differentiation into Cardiomyocyte

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Objective: Development of science and producing new technology are two important factors which playing main role in inventing new medical treatments for important diseases, like critical heart problems. Heart failure which becomes common in our world, due to increasing the rate of obesity and lacking physical exercises in people lifestyles, is produced by inability of cardiomyocytes in self-renewal. So, using targeted differentiation from mesenchymal multi-potent stem cells into cardiomyocytes could be the sure way for curing this medical condition. Because of this, we focused on finding new pathway for more effective and efficient stem cell targeted differentiation.
Materials and Methods: In this experiment, we used Adipose tissue derived stem cells. The extraction method is using collagenase type I on highly fragmented tissue from midscapulare of male rabbit. Flowcytometry used for characterization of extracted cells. Additionally, for examining the multi-potency of these stem cells, the osteogenic and chondrogenicm differentiation have been done. The collagen coated membrane in chemical, mechanical and chemical-mechanical pathways have been used as an appropriate surface for differentiating. The undifferentiated Rabbit stem cells are negative control and cardiomyocytes used as positive one. The interval between affecting stem cells and RNA extraction in our experiments is 4 days. We set GATA 4 (cardiac-specific transcription factor) as target gene besides the HPRT (housekeeping gene) for surveying in Taqman base real time-PCR.
Results: The multi-potency of extracted stem cells are approved by the results of osteogenic and chondrogenic differentiation and flocytometry data shows stem cells' specification. The data from real time-PCR is clearly approved that the mechanotransduction have significant effect on differentiation process by increasing the expression of GATA 4 in treated cells.
Conclusion: According to the information that mentioned in results, mechanical loading could be the more appropriate and effective way for targeted differentiation in comparison with chemical factors. Also, the combination of mechanical loading and chemical factors may have more effective influence on adipose derived stem cells for differentiating to cardiomyocyte than other two pathways.

Keywords: Mesenchymal Stem Cell, Cardiomyocyte Mechanotransduction, Taqman Base Real Time-PCR

Ps-117: Quantitative Proteomics Analysis of Neural Cells Differentiated from Human Embryonic Stem Cell

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Objective: In the early stages of embryonic development, cell commitment occurs by thousands of genes and proteins; nevertheless quantity of each protein is crucial. In the most simplified model of human CNS development, human embryonic stem cells (hESCs) could develop into the neural cells *in vitro*.
Materials and Methods: In this study, quantitative proteomics of hESCs investigated during neural commitment and differentiation by 2D-DIGE coupled MS/MS.
Results: we found that expression of 137 protein spots modulated during differentiation, of them 118 proteins could be identified using MALDI-TOF/TOF and LC MS/MS. Several proteins involved in mRNA processing, protein folding and actin related proteins down regulated whereas Peroxiredoxins (anti oxidative stress proteins), anti apoptosis proteins, cell structure and motility proteins were up regulated in neural progeny. Gene expression, cell death, replication and recombination pathways connected in a significant manner to the hESC specific proteins and neural associated proteins were involved in the cell death, cell maintenance and cancer pathways. Functional analysis figured up that suppression of MAGOHB protein in hESCs enhanced the neural differentiation and hindered other lineages development by unknown mechanisms. Also we found that BCAS2 expression is mandatory for stem cells pluripotency and its knock down promotes differentiation through p53 signaling pathway.
Conclusion: Current study provides us comprehensive source of proteins that affect neurogenesis *in vitro* and shed light on protein networks with a unknown functions in neutralization.

Keywords: 2D-DIGE, BCAS2, Neural Development, HESCs, Proteomics

Ps-118: Effects of PPARγ Activation and Inactivation on Colonies Formation from Single Human

Embryonic Stem Cells Treated with Rock Specific Inhibitor

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Objective: Although hESCs are pluripotent cells derivatized from the blastocyst embryo like mESCs, there are several considerable differences between them. Massive cell death occurs after complete single-cell dissociation because hESCs are very vulnerable to single-cell dissociation. Massive hESCs death is due to Activation of Rho/Rock pathway and using Rock Specific inhibitor (Y-27632) permits the survival of hESCs by blocking the dissociation-induced cell death. PPARγ is a nuclear receptor that has many functions in different cells. One of the most important roles of this nuclear receptor is anti-apoptotic function. Moreover, our preview studies showed that this nuclear receptor involved in mESCs proliferation. So, in this study we were going to show whether inhibition of Rock pathway to survive hESCs could have relation with this nuclear receptor or not
Materials and Methods: To study effects of Rock signaling pathway inhibition on PPARγ expression, hESCs were dissociated and simultaneously 10μM Rock Specific inhibitor (Y-27632) was added. After 1, 2, 4 and 6 hours, cultured cells were harvested and Real-Time PCR was applied to demonstrate effects of Rock signaling pathway inhibition on PPARγ expression in compared with untreated (without Y-27632 treatment) cells. Also, to clarify the role of PPARγ in this process, PPARγ agonist (Pioglitazone) and antagonist (GW9662) were added when PPARγ expression level had been increased under Rock inhibitor induction. After 2-3 days Colony formation assay was perfumed with Alkaline Phosphatase (AP) test
Results: Real-time PCR showed increasing PPARγ expression level due to inhibition of Rock signaling pathway (with Y-27632) after 2 hours treatment. Simultaneous using PPARγ agonist with Rock inhibitor moderately increased colony formation, however using GW9662 decreased colony formation
Conclusion: These data showed PPARγ probably involved in cell signaling pathway of colonies formation from single human embryonic stem cells in presence of Rock inhibitor
Keywords: Rock Inhibitor, PPARγ Agonist and Antagonist, Human Embryonic Stem cells, Colony Formation

Ps-119: Expression Analysis of "Putative Stemness" Jam2 Gene in Gastric Adenocarcinoma and Normal Adjacent Tissues

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Objective: Recent microarray analyses have led to the identification of putative "stemness" genes that are expressed in ESCs, NSCs,and HSCs but not in their differentiated derivatives. jam2,one of these genes, is one of the protein components in junction adhesion molecules. In this study we have analyzed the expression level of this gene in gastric adenocarcinoma and normal tissues. our goal is to study the role of this gene in tumor progression.
Materials and Methods: 20 pairs of tumor and adjacent normal tissues were collected. their RNAs were extracted and after their analysis cDNA synthesis were done. Real time PCR was done and the result was statistically analyzed.
Results: The expression level of the jam2 in tumor tissues was lower than normal adjacent tissues. this difference was significant ($p = 0.02$)
Conclusion: This may support the hypothesis that the junctional complex disrupts during the progression of the cancer in order to metastasis.
Keywords: Stemness, Jam2, Adenocarcinoma, Eexpression

Ps-120: Mouse Bone Marrow Mesenchymal Stem Cells (mBMSCs) Differentiation into Neural-like Cells by Staurosporine together with Morphine

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Objective: Mouse bone marrow mesenchymal stem cells (mBMSCs) can be differentiated into neural-like cells under appropriate experimental conditions. This study was designed to introduce a new protocol to neural differentiation based on staurosporine and morphine applications on neural differentiation in mBMSCs.
Materials and Methods: Mouse BMSCs were isolated and cultured in DMEM containing 10% FBS. Differentiation assay and RT-PCR for rex-1 were done to affirmation multipotency of isolated cells. Cells were treated with 0, 50, 100, 214 and 316 nM of staurosporine (treatments I, II, III, IV and V; respectively) without

morphine (group 1), and with 10-4 morphine (group 2). Neutral red uptake and total neurite length in each group were assessed 6, 12 and 24 hours after treatments. RT-PCR for MAP-2 and immunostaining for GFAP, MAP-2 and Tub β-III were used to affirmation neural differentiation in BMSCs after treatment with differentiation medium containing staurosporine and morphine.
Results: Cells were differentiated into osteocytes and adipocytes after exposure to special differentiation media. RT-PCR showed that isolated cells express rex-1 as a stem cell marker. Results showed that the viability of cells in group 2 in all treatments is higher than the same treatments in group 1 ($p<0.05$). Neurite outgrowth was increased in group 2 compared with group 1. In two groups viability of cells in all treatments was reduced from 6 to 24 hours but severity of viability decreasing in group 2 was lower than group 1($p<0.05$). RT-PCR and immunostaining showed that the cells expressed neural markers after treatment with neural differentiation medium containing staurosporine and morphine.
Conclusion: Our results suggest that staurosporine together with morphine can be used as a neural differentiation inducer in differentiation studies.
Keywords: Staurosporine, Morphine, Neural Differentiation, BMSC, Mouse

Ps-121: The Combination of Shear Stress and Hydrostatic Pressure Modulate Chondrogenic Induction of Human Adipose Derived Mesenchymal Stem Cells

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Objective: In this study, we examined how epigenetic factors such as the biomechanic environment govern stem cell fate. Furthermore, we hypothesize that bioengineered substrates and high-density cell culture systems are leading keys to control chondrogenic induction of the mesenchymal stem cells (MSCs).
Materials and Methods: To test these hypotheses, human adipose derived mesenchymal stem cells (hASCs) were exposed to 1 Pa shear stress and 3 MPa hydrostatic pressure for 60 minutes on chemically designed medical grade HTV silicone rubber, while no slouble growth factors were added to the culture medium. Transcriptional profiles for terminal differentiation markers of hADSCs were monitored by real time PCR analysis of monolayer cultures.
Results: Gene expression patterns were dependent on the loading regime used in well controlled *in vitro* model system. A greater effect on collagen type II (ColII) mRNA expression was observed for in group C and E compared to the control (group A). Upregulation of aggrecan (Agg) mRNA was similar to the observations in ColII expression profiles.
Conclusion: Recently MSCs can differentiate into articular cartilage in an aggregate forming culture systems (pellet and micromass), which need culture manipulation and are difficult to use conventional imaging technique. We present a novel method for generation of hASC-derived chondrocytes in monolayer cultures under mechanically defined conditions. By looking at gene expression patterns it could be concluded that Wnt and TGFβ signaling pathways are involved in chondrogenesis caused through imposing the combination of shape changing deviatoric shear stresses and volume changing cyclic hydrostatic pressure. In addition, both hydrostatic pressure and shear stress alone was insufficient to induce chondrogenic response in hASCs cultures. However, the application of shear stress and hydrostatic pressure led to enhanced chondrocyte specific genes.
Keywords: Adipose Derived Mesenchymal Stem Cell, Shear Stress, Hydrostatic Pressure, Chondrogenesis

Ps-122: Effects of Combination of Mechanical and Chemical Factors on Osteogenic Differentiation of Mesenchymal Stem Cells

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Objective: Cells are exposed to mechanical forces of different types every day and respond to these mechanical loads with changes in their structure and function. Functional changes include growth, gene expression pattern, protein synthesis, differentiation and cell movement. Mesenchymal stem cells (MSCs) are undifferentiated and prone to convert to osteoblast, myoblast, chondrocyte and adipocyte. So these cells have been selected for tissue engineering and regenerative medicine. Mechanical stimulation has been shown to promote osteogenic differentiation of MSCs.
Materials and Methods: Bone marrow-derived stem cells were isolated from a rat and cultured in α-MEM medium containing 10% FBS. Multipotential property of cells was examined by incubating cells in chondrogenic and adipogenic mediums and flow cytometry method was performed to verify the purity of isolated cells. Four test groups were assigned in this research: mechanical, chemical, mechanical/chemical and control groups. In mechanical group MSCs were seeded on col-

lagen I coated membrane and exposed to cyclic uniaxial stretch of 3% and 0.3 Hz frequency for 24 hours by a cyclic stretch device fabricated in National Cell Bank of Iran. In chemical group cells were treated with culture medium supplemented with 1 μM dexamethasone, 10 mM β-glycerophosphate and 0.1 mM ascorbic acid for 10 days. In mechanical/chemical group, cells were subjected to the strain for 24 hours and then cells were incubated in osteogenic medium for 10 days. Real-Time PCR carried out to evaluate the expression of osteogenic marker genes (Runx2 and OCN).
Results: Real-time results showed the expression levels of Runx2 and OCN were much more increased in mechanical/chemical group in comparison to mechanical and also chemical ones.
Conclusion: Combination of mechanical strain and chemical factors is much more suitable for directing osteogenesis and these two elements enhance each other.
Keywords: Stem Cell, Differentiation, Osteoblast, Mechanical Loading

Ps-123: Roles of The Injection of Fibroblast Growth Factor-2 and Neuregulin-1 on Stimulation of Stem Cell Migration on Sub Ventricular Zone

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Objective: Progenitor cells in the sub ventricular zone of the lateral ventricle can proliferate throughout the life of the animal. Neurons progenitor cells are highly sensitive to ischemic strokes.
Materials and Methods: Recent evidence points to a relationship between neuregulin-1(Nrg-1) and the process of neural cells differentiation. Fibroblast growth factor-2 (FGF-2) and its multiple high-affinity FGF receptors (FGFRs) are regulating neural differentiation and migration mainly by controlling precursor cells proliferation. This study was aimed to evaluate the combination of FGF-2 and Nrg-1 sub ventricular zone injection on progenitor cells migration. Middle cerebral artery (MCA) Ischemic stroking and precursor cells labeling by injection of bromodeoxyuridine (BrdU) in sub ventricular zone were suggested.
Results: A remarkable increase expected 4 weeks after injection of FGF-2 and Nrg-1 factors in the number of labeled cells in olfactory bulb by immunohistochemistry method.
Conclusion: On the basis of these findings, we hypothesized that simultaneous injection of FGF-2 and Nrg-1cause enhancement on the amount of sub ventricular zone precursor cells migration.
Keywords: Sub Ventricular Zone, Fibroblast Growth Factor-2, Neuregulin-1, Stem Cell Migration

Ps-124: Cyclic Stretch Stimulates Myh2 Expression in Differentiation of Mesenchymal Stem Cells into Skeletal Muscle Cells

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Objective: Skeletal muscle cells have the potential to respond to environmental factors including biomechanical signals. Cyclic stretch is one of the important factors affecting differentiation of mesenchymal stem cells (MSCs) into skeletal muscle cells .The aim of this study was to illustrate the effect of environmental factors including chemical cues and cyclic strain on myogenic differentiation of MSCs by evaluation of myh2 expression.
Materials and Methods: This study was designed within 5 days in 4 groups for MSCs: control, chemical, chemical-mechanical and mechanical groups based on BMSCs, after isolation from mouse. In chemical tests, MSCs were exposed to chemical growth factors, 5-azacythidine and horse serum, for 5 days. In mechanical tests, a cyclic axial stretch device, developed in National Cell Bank of Pasteur Institute of Iran, was used for mechanostimulation. MSCs cultured on collagen type1 coated silicon membrane were subjected to cyclic stretch (8% strain, 1 Hz freq. and 24 hours). In chemical-mechanical tests, MSCs were loaded for 24 hours after they were treated by differentiation medium. MSCs were used in negative control group. In order to investigate the myogenic differentiation of MSCs Real-Time PCR was used to examine the expression of skeletal muscle cell specific gene, myosin heavy chain 2 (Myh2). Immunocytochemical staining with skeletal muscle cell antibody, Myh, was carried out in order to detect the presence of skeletal muscle cell marker.
Results: According to the results, chemical-mechanical groups showed a higher expression of Myh2 (20.7 folds) in comparison to mechanical (8.94 folds), and also chemical (5.2 folds) ones.
Conclusion: These Results suggest that uniaxial cyclic strain alone can affect myogenic differentiation of MSCs. The combination of chemical with mechanical factors stimulates differentiation much more than differentiation by chemical factors or mechanical signals alone and produces functional skeletal muscle cells for engineered tissues.
Keywords: Stem Cells, Differentiation, Cyclic Stretch, Skeletal Muscle Cells

Ps-125: Human Fresh Frozen Plasma (FFP) for Isolation and Expansion of Wharton's Jelly Mesenchymal Stem Cells for Clinical Use

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Objective: Mesenchymal stem cells (MSC) are promising candidates for cell-based therapies. One major obstacle for their clinical use is the biosafety of fetal calf serum (FCS), which is a crucial part of currently used media for the culture of MSC.
Materials and Methods: UC-MSC was isolated by cutting the umbilical cord into 1 mm3 fragments and then put the fragments in α-MEM medium with 10% FBS, filtered FFP (10%, 5%, 3% and 1%), filtered and inactivated FFP (10%, 5%, 3% and 1%) respectively. UC-MSCs express the pluripotency markers Oct-4, Sox-2, and Nanog by PCR. The cells were CD90, CD105 and CD44 positive by flow Cytometry and could be induced to mesodermal lineages including osteogenic and adipogenic differentiation.
Results: Isolation of MSC after 3 days appeared best in media containing 10% FBS and after that with 1% filtered FFP. The medium of other concentration of FFP showed gelatinous change and the cells finally detached from the plate.
Conclusion: One percent filtered FFP appears at least as good as 10% FCS with regard to both isolation and expansion of human MSC, while 5%,3% and 10% appear inferior.
Keywords: FFP, Wharton's Jelly, Mesenchymal Stem Cells, Clinic

Ps-126: Efficiency of Dendrimer G6 in FITC Conjugated Micro RNA Transfection to Embryonic Stem Cells, Unrestricted Somatic Stem Cells and Mesenchymal Stem Cells

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Objective: Gene delivery vectors are divided into two groups: viral and non-viral vectors. Viral vectors are efficient in delivering of a gene to the target cells although, there are problems related to the safety and large scale production. So, non-viral vectors including of chemical vectors are still attractive because they are not immunogene, so they are more safe and easy for preparation. Among chemical vectors, dendrimers are a system used for gene delivering into cells. In this study, efficiency of dendrimer G6 (as a nucleic acid porter) was assessed in transferring of scramble microRNA-FITC conjugated into human mesenchymal stem cells; cord blood derived unrestricted somatic stem cells (USSC) and mouse embryonic stem cells, in comparison to lipofectamine.
Materials and Methods: To asset to a real statitistic range for micro RNA (miR) transfection, the cell transfected by FITC conjugated micro RNA. After 24, 48 and 72 hours the transfection efficiency was investigated by fluorescent microscopy, flow cytometry and MTT assay.
Results: Flourescent microscopy showed green color inside the cells. Flow cytometric studies showed G6 has higher transfection efficiency in hMSCs, USSCs and the embryonic stem cells than lipofectamine. Although, cytotoxicity studies by MTT revealed that dendrimer G6 had a higher cytotoxicity effect than lipofectamine in miR transferring to all of the given cells.
Conclusion: Therefore, in respect to efficiency of gene transferring and cost, our study introduces dendrimer G6 as an efficient carrier for miR transferring to different types of stem cells.
Keywords: G6 Dendrimer, Lipofectamine, Gene Transfer, Mesenchymal Stem Cells, Embryonic Stem Cells, Unrestricted Somatic Stem Cells

Ps-127: The Assessment of CD133 Expression in Human Melanoma Cell Line

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Objective: Melanoma is most dangerous form of skin cancer. This cancer is responsible for almost 60% of lethal skin tumors. Melanoma originates from normal pigment cells called melanocytes. CD133, a transmembrane glycoprotein, is considered the most important cancer stem cell (CSC)-associated marker identified so far. The aim of this study is characterization of sorted CD133+ and CD133- cells as cancer stem cells.
Materials and Methods: D10 melanoma cell line was grown in complete RPMI medium. The cells were sorted up on the expression of CD133 as CD133-postive and CD133-negative population. The potential of colony formation, spheroid formation, and invasion

were assessed in sorted groups separately. The paired two-tailed Student's t test was used to compare groups and P value less than 0.05 was considered statistically significant.
Results: The CD133 was expressed in approximately 30% of D10 cells. The colony formation potential was significantly higher in CD133+ cells than CD133- cells (3-4 folds). Also, the number of spheroid increased in CD133+ cells vs. negative part (2-3 folds). However there is no difference in invaded cells between two groups ($p \geq 0.05$).
Conclusion: our result support that CD133+ cells in melanoma can a candidate for cancer stem cell population. However exactly detection of cancer stem cell required more assay such as assessment of expression of stemness genes, and also injection of this cells to NOD-SCID mice and determination of tumorogenicity.
Keywords: Melanoma, CD133, Cancer Stem Cell

Ps-128: *In Vitro* Expression of Insulin Like Growth Factor I Receptor (IGF-IR) on Human Bone Marrow Derived Mesenchymal Stem Cells Induced by TNF-α

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Objective: Acute hepatic failure (AHF) is a severe liver injury accompanied by sustained liver damage. Management of severe AHF is one of the most challenging problems in clinical medicine . It is imperative that new approaches for repairing the liver are developed. Cell-based therapy has been implicated in the treatment of liver diseases. Mesenchymal stem cells (MSCs) from various sources such as bone marrow is available. These cells is one of the major candidates in cell therapy. The production of IGF-I increases in the regenerating liver. Insulin-like growth factor – I (IGF-I) in liver regeneration is effective after binds to IGF-I receptor. Increasing expression of IGF-IR in tumor necrosis factor-α (TNF-α) - treated MSCs may be cause to improve liver regeneration.
Materials and Methods: Bone marrow was aspirated from human normal donor after inform consent. Cells were isolated and cultured. Identification of cells with Flow cytometric analysis and functional testes were performed. Fourth passage cells were treated with TNF-α at different doses (1ng/ml and 10ng/ml) and incubated at different times (2,10,24 and 48 hours). IGF-IR gene expression was investigated Using Real time - polymerase chain reaction technique.
Results: Flowcytometric analysis showed that MSCs towards CD90 marker positive while is negative with respect to CD45,CD80, CD40 markers. Functional test for MSC was demonstrated by adipocyte and osteocyte-staining with Oil- Red and Alizarin Red respectively. Increased gene expression was demonstrated in TNF-α treated in comparison with untreated cells.
Conclusion: Increase gene expression pattern of IGF-IR in human MSCs may be used for clinical stem cell therapy in AHF.
Keywords: Acute Hepatic Failure, Mesenchymal Stem Cells, Insulin-like Growth Factor -I Receptor

Ps-129: Comparison between Mouse Embryonic Fibroblast (MEF) Yield from 13 Day Old and 17 Day Old BALB/C Mice for Culture and Reproduction of Induced Pleuripotent Stem Cells

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Objective: In order to cultivate and produce embryonic stem cells (ESC) and induced pleuripotent stem cells (iPS), we need a cellular bed. The best condition is offered by fibroblast of the mouse embryo. During inactivation, cell division is stopped and only their metabolic activities continue and support the stem cells survival.
Materials and Methods: In the present study 13-day-old and 17-day-old embryos from pregnant female BALB/C mice were used. Material needed for the experiment include; high glucose DMEM medium supplemented with 10% FBS, 0.1 mM mercaptoethanol, 2 mM L-glutamin, 100 IU/ml penicillin/streptomycin. Fragments of the embryo abdominal wall were cultured as explants in 10cm culture plate already covered with 0.1% gelatin.
Results: In comparison, 17-day-old embryo had higher density of MEF through explants. Fragments taken from 17-day-old embryo body began migration in less than 24 hours after cultivation and became confluent 3 days after migration
Conclusion: To produce MEF cells for cultivation and reproduction of Esc and iPS, 17 day old mouse embryo is much better than 13 day old one, because the cells leave the tissue in a shorter period of time and they become more confluent in subculture with better quality when compared with the cells obtained from 13-day-old embryo.
Keywords: MEF, Culture, Stem Cell

Ps-130: Comparative Investigation of The Osteogenic Differentiation Potential of Different Mesenchymal Stem Cells

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Objective: Recently cell-based therapy approach seems to be a promising procedure for regeneration of bone defects. However finding the best cell source is challenging and every cell types have their pros and cons.
Materials and Methods: In this study, the biomedical characteristic and osteogenic capability of the three stem cell groups containing mesenchymal stem cells isolated from bone marrow (BM-MSC), cord-blood-derived, unrestricted somatic stem cells (USSC) and adipose tissue (AT-MSC) are proposed as potential sources for cell therapy and functional tissue engineering.
Results: The proliferation rate of the three named stem cells proliferation is the same to a great extent ($p \leq 0.05$). In osteogenic differentiation, alkaline phosphatase (ALP) activity reached the highest point in day 7 in unrestricted somatic stem cells, in comparison to mesenchymal stem cells isolated from bone marrow (BM-MSC) which displayed the most significant value of ALP activity in day 14. In whatever manner, during osteogenic induction, BM-MSC revealed the maximum ALP activity and mineralization .Moreover, adipose tissue stem cells presented the least capability for mineralization within differentiation and had the lowest ALP activity on days 7 and 14. Even supposing, the rate of expression for levels of osteonectin , collagen type I, and BMP-2 in undifferentiated state AT-MSC was significantly higher, but these genes were expressed sharply in BM-MSC during differentiation. In the incubation process, BM-MSC expressed the greatest amount of ALP, Runx2, and osteocalcin.
Conclusion: All things considered, BM-MSC included the greatest potential for osteogenic differentiation and keep a valuable treasure in cell based therapies and tissue engineering applications.
Keywords: Stem Cell, Bone, Differentiation, Cell Therapy

Ps-131: Induction of Pluripotency in Human Skin Keratinocytes by Plasmid Containing Two Transcription Factors Oct4 and Sox2

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Objective: Reprogramming differentiated human cells to induced pluripotent stem (iPS) cells has applications in basic biology, drugs development and transplantation. It has been established that ectopic expression of four transcription factors Oct4, Sox2, c-Myc and Klf4 in fibroblast cells. The aim of this study was induction of pluripotency in human skin keratinocytes using plasmid containing two transcription factors Oct4 and Sox2.
Materials and Methods: In the present study, keratinocyte cells isolated and cultivated from human foreskin. Gene transfer process by plasmid containing GFP gene (pCAG-GFP) was optimized. For cell transfection used from cationic lipid effectene. iPS cells were then established using pSIN4-EF2-O2S plasmid vector and characterized using embryonic stem cell markers.
Results: Transfection of cells, using the two transcription factors, indicated that reprogramming occurs gradually in a process 2-3 weeks, markers of pluripotency for example ES cells specific antigens and genes and alkaline phosphatase enzyme activity are detected in reprogrammed cells.
Conclusion: The iPS cells that were generated using plasmid vector in this study could be used as a valuable instrument for the studing the iPS cells and mechanisms of cell reprogramming. These studies, on one hand can extend our understanding on the mechanism of controlling genome and cell differentiation, on the other hand, such studies may open up new avenues for safe clinical application using iPS cells.
Keywords: Reprogramming, iPS Cells, Keratinocyte, Plasmid

Abstract of Precongress Workshop

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Mesenchymal Stem Cells: Isolation, Purification and Differentiation

Mesenchymal stem cells (MSCs) are defined as non-hematopoietic cells that are able to replicate for a long time while maintaining their multilineage differentiation potential.
MSCs are considered as a suitable candidate for cell therapy strategies owing to their ability to undergo extensive proliferation and their potential to undergo differentiation into different cell lineages. Their efficacy has been indicated in curing osteogenesis imperfecta, regenerating bone and cardiac muscle and resurfacing articular cartilage as well as restoring hematopoiesis in patients receiving chemotherapy. The potential of MSC in differentiating into cells other than those of skeletal lineages, such as neurons and keratinocytes as well as liver, intestine and kidney epithelial cells has also been demonstrated by several experiments.
In this workshop, the main components that are involved in cell preparation for therapy including cell isolation and culture, evaluation of differentiation potential of extracted cells and quality control of cells of aspects such as growth rate and viability will be trained.

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