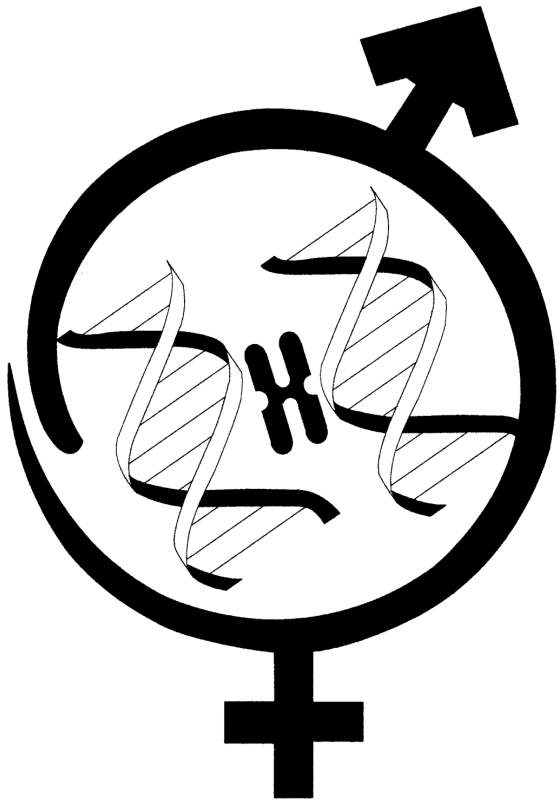
Abstracts of

Royan International Twin Congress

11th Congress on Stem Cell Biology and Technology

2-4 September 2015



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Cell Journal(Yakhteh)

Guide for Authors

Aims and Scope: The "***Cell Journal*** *(Yakhteh)*" is a quarterly English publication of Royan Institute. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on exclusively Cellular, Molecular and other related topics. ***Cell J***, has been certified by Ministry of Culture and Islamic Guidance since 1999 and also accredited as a scientific and research journal by HBI (Health and Biomedical Information) Journal Accreditation Commission since 2000. This journal holds the membership of the Committee on Publication Ethics (COPE).

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Dr. Saeed Kazemi Ashtiani

IN THE NAME OF GOD

Gone But not Forgotten

In the memory of the late Director of Royan Institute, Founder of Stem Cells Research in Iran and Chairman of ***Cell Journal*** *(Yakhteh)*. May he rest in peace.

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Contents:

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

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Yaser Tahamtan

Dear Colleagues and Friends,

On behalf of organizing committee, it's my great pleasure to welcome you to the 11th International Congress on Stem Cell Biology and Technology which is concurrently held with 16th congress on Reproductive Biomedicine (Royan International Twin Congresses), on September 2-4, 2015.

Royan Institute for Stem Cell Biology and Technology (RI-SCBT) is one of the leading institutes conducting basic and translational researches on stem cells, developmental biology and regenerative medicine. Therefore, RI-SCBT is playing fundamental role in creating an opportunity for scientists in the field of cell biology and regenerative medicine as well as physicians to address the latest developments and exchange their findings in a scientific and vibrant atmosphere. To achieve this goal, we annually hold this scientific meeting as of 2005.

Increasing number of participants and high volumes of articles all indicate this fact that this field of research is on progress. About 800 participants including enthusiastic young researchers and distinguished investigators throughout the world have taken part in this annual event. In the present congress (11th International Congress on Stem Cell Biology and Technology), 13 international invited speakers from 11 various countries as well as more than 90 poster presenters discuss latest progress in different fields including pancreas and diabetes, neural and retinal diseases, animal disease modelling, tissue engineering, cell therapy, pluripotent stem cells, cancer and personalized medicine. One of the main features of the present congress is the emphasis on interdisciplinary aspects of regenerative medicine which gathers biologists, physicians and engineers together.

Hope to see you in Tehran, Iran

Yaser Tahamtan, Ph.D.
Congress Chairman of 11th Congress
on Stem Cell Biology and Technology

Is-1: Antigenicity and Immunogenicity of Prostate Cancer Stem-Like Cells

Bellone M

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Objective: While consistent evidence supports the concept that, irrespective of the cell-of-origin, many cancers are hierarchically organized as a normal tissue, and therefore, they contain stem-like/progenitor cells (CSCs), whether CSCs are susceptible to immune surveillance is poorly defined. We addressed this biological problem in a mouse model of prostate cancer.
Materials and Methods: Utilizing the sphere assay, we established long-term prostate CSC lines from unsorted prostate cells obtained from different stages of prostate cancer progression in transgenic adenocarcinoma of the mouse (TRAMP) mice, which develop autochthonous prostate lesions evolving from mouse prostate intraepithelial neoplasia (mPIN) to NE differentiation. CSCs lines were assessed *in vitro* and *in vivo* for their antigenic content and immunogenicity, i.e., the ability to induce a specific immune response.
Results: Prostate CSC lines expressed prostate cancer associated antigens, MHC I and MHC II molecules and ligands for natural killer (NK) cells. Indeed, CSCs were targets of NK and cytotoxic T lymphocytes both *in vitro* and *in vivo*. Vaccination with dendritic cells pulsed with irradiated CSCs induced a tumor-specific immune response that was stronger than the one induced by dendritic cells pulsed with differentiated tumor cells, delayed tumor growth in mice challenged with prostate CSCs, and caused tumor regression in TRAMP mice.
Conclusion: Thus, CSCs are targets of both innate and adaptive immune responses and might be exploited for the design of novel immunotherapeutic approaches against cancer.

Is-2: Role of Extracellular Matrix Proteins in Cancer Cell Dormancy within Prostate Cancer Draining Lymph Nodes

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Objective: Precociously disseminated cancer cells may seed quiescent sites of future metastasis if they can protect themselves from immune surveillance. However, there is little knowledge about how such sites might be achieved. We wanted to investigate this issue in a preclinical model of prostate cancer.
Materials and Methods: Utilizing the sphere assay, we established long-term prostate cancer stem-like/progenitor cell (CSC) lines from unsorted prostate or prostate draining lymph node cells obtained from different stages of prostate cancer progression in transgenic adenocarcinoma of the mouse (TRAMP). CSCs lines were assessed *in vitro* and *in vivo* for immunosuppressive activities.
Results: CSCs were found in histopathologically negative prostate draining lymph nodes in TRAMP mice harboring oncogene-driven prostate intraepithelial neoplasia (mPIN). Prostate draining lymph node-derived CSCs were phenotypically and functionally identical to CSCs obtained from mPIN lesions, but distinct from CSCs obtained from frank prostate tumors. These CSCs blocked T cell functions *in vitro* and *in vivo* through several mechanisms, including the release of extracellular matrix proteins like Tenascin C (TNC) and Galectin-3 (Gal-3). CSCs from both PDLN and mPIN lesions also expressed CXCR4 and migrated in response to its ligand CXCL12, which was overexpressed in prostate draining lymph nodes upon mPIN development. CXCR4 was critical for the development of prostate draining lymph node-derived CSCs, as *in vivo* administration of CXCR4 inhibitors prevented establishment in PDLN of an immunosuppressive microenvironment.
Conclusion: Taken together, our work establishes a pivotal role for TNC and other factors released form CSCs in fine-tuning the local immune response to establish equilibrium between disseminated nodal CSCs and the immune system.

Is-3: RPE Replacement by Transplantation of Human Embryonic Stem Cell-Derived RPE in Rodent Models of Retinal Degeneration

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Objective: Retinal diseases affecting the retinal pigment epithelium (RPE), like age-related macular degeneration, Stargardt disease or Leber's congenital amaurosis, represent a leading cause for visual impairment and blindness with no cure currently established. Transplantation of RPE is discussed as a potential therapy for the replacement of degenerated RPE cells. Here we assessed the use of human embryonic stem cell- (hESC) derived RPE cells for transplantation into two rodent models of RPE dysfunction and loss.
Materials and Methods: RPE cells were generated *in vitro* from hESCs and sub-retinally transplanted into Royal College of Surgeons (RCS) rats and wild-type mice systemically injected with sodium iodate. Sham- and fibroblast-injected animals served as controls. Experimental animals were analysed by immunohistochemistry, RT-PCR, electron-microscopy and the

thickness of the outer nuclear layer was quantified.
Results: RCS rats carry a spontaneous mutation in the Mertk gene leading to dysfunction of outer segment phagocytosis and photoreceptor degeneration. Following transplantation into RCS rats donor RPE cells mainly formed clusters in the subretinal space beside few small monolayer structures. Transplantation of hESC-derived RPE cells resulted in a significant thicker ONL in comparison to untreated or sham-injected hosts. However, transplanted fibroblasts showed a similar protective effect. Injection of sodium iodate caused complete RPE cell loss, photoreceptor degeneration, and altered gene and protein expression in outer and inner nuclear layers. Following transplantation, donor hESC-derived RPE cells formed extensive monolayers that displayed wild-type RPE cell morphology, organization, and function, including phagocytosis of host photoreceptor outer segments.
Conclusion: RPE cells derived from hESCs survive for considerable time periods within rodent models of retinal degeneration. Cluster formation and comparable rescue levels propagated by transplanted fibroblasts questions specific RPE function causative for the protective effects observed in RCS rats. Systemic injection of sodium iodate has considerable effects on RPE, photoreceptors, and inner nuclear layer neurons, and provides a model to assay reconstitution and maturation of RPE cell transplants.The availability of an RPE-free Bruch's membrane in this model likely allows the unprecedented formation of extensive polarized cell monolayers from donor hESC-derived RPE cell suspensions.

Is-4: Epiphyseal Chondro-Progenitor Cells for The Treatment of Full Thickness Cartilage Injuries – An Experimental GLP-grade Safety Study in Goats

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Objective: bone marrow stimulation or microfracture (MF) is the most widely used surgical technique to repair cartilage tissue injuries. While repair tissue is built relatively quickly, the MF procedure leads to the formation of a fibrocartilagenous scar tissue. Another major pitfall of MF is the instability of the mesenchymal blood clot formed. Autologous Matrix Induced Chondrogenesis (AMIC) aims stabilize the blood clot with a membrane, which can be glued in place using tissue sealant. The clinical superiority of AMIC compared to standard MF however has not yet been determined by randomized trials. As such, we have endeavored to combine the matrix-stabilizing technique with bioactive factors, namely therapeutic cells, which may effectively interact with reparative cells from the bone marrow and transform the repair response to a regenerative response. We have previously reported the reliable expansion and characterization of a clinical-grade human epiphyseal chondro-progenitor (ECP) cell bank from a single tissue donation. Aimed for allogenic off-the-shelf implantation, ECPs exhibited remarkable homogeneity and stability in expansion. We have conducted a GLP-grade pre-clinical safety study in goats to assess the effect of implanted ECPs in a full thickness cartilage defect. ECPs were delivered within a collagen-based matrix. The cell-laden construct is delivered in combination with MF to direct new tissue repair and remodeling. We present here the findings from our 3-months pre-clinical study, focusing on the safety of ECPs, the feasibility of the proposed treatment protocol as well as early indications of repair.
Materials and Methods: This study was performed in compliance with Principles of Good Laboratory Practice (OECD, C(97)186/Final). All animal experiments were conducted according to Swiss laws of animal protection and welfare and authorized by the cantonal ethical committee (license 174/2012). Eight female Saanen goats were randomized to two treatment groups. Six goats in the "ECP" group received ECPs seeded in Chondro-Gide® collagen matrix (Geistlich, Switzerland) over MF in full thickness chondral defects. Two goats in the "CTR" group received saline soaked Chondro-Gide® matrix over MF. Full thickness chondral defect were performed in medial and lateral condyles of the stifle joint. Animals were sacrificed 3 months after the surgery. Organ and tissue samples were processed to screen for traces of human cells as well as potential histological abnormalities. Magnetic Resonance Imaging (MRI) was performed on operated stifles to detect subchondral bone sclerosis and bone marrow edema. Macroscopic, histological and immunohistochemical assesments were performed to evaluate the quality of early repair as well as that of the surrounding cartilage and subchondral bone.
Results: The macroscopic state of repair as well as subsequent subchondral bone sclerosis and bone marrow edema showed no statistically detectable difference compared to control. A trend was however observed in cartilage tissue surrounding the defects with the ECP group exhibiting healthier, better preserved surrounding cartilage than that in the CTR group. Tracking ectopically engrafted human cells did not reveal pathological inclusions.
Conclusion: ECP implantation in combination with AMIC may provide necessary protective cues to maintain joint homestasis during repair. Whether ECPs are more likely to work as chaperones of repair or builders of new tissue, results from the 3-months study highlights their safety. The proposed implantation protocol provides the operating room staff with enough flexibility without requiring much pre-planning or the need for

additional expensive equipment in the operating room. The results obtained will help inform the design of a long-term investigation attempting to achieve functional cartilage regeneration.

Is-5: Generation of Functional Islets of Langerhans from Human Exocrine Pancreas

Docherty K

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Objective: Cell therapy in the form of human islet transplantation has been a successful form of treatment for patients with type 1 diabetes for over 15 years, but is significantly limited by lack of suitable donor material. During the islet isolation procedure, 98% of the pancreas, comprising acinar and ductal tissue (the exocrine tissue) is normally discarded. The aim of this study was to optimize a protocol whereby this exocrine material could be efficiently reprogrammed to provide an additional supply of islets for transplantation.
Materials and Methods: On arrival in the lab the exocrine-enriched fraction was immediately plated on to tissue culture dishes. The cells attached to the dish and formed a monolayer, which if left unchecked would undergo the epithelial to mesenchymal transition (EMT). This mesenchymal monolayer could be repeatedly passaged. During preliminary experiments, we found that inhibiting the process of EMT (day0 – day1) using TGFBeta1 and Rho kinase inhibitors enhanced the efficiency of reprogramming. The cells were also treated with chromatin modifying agents at this stage. Reprogramming was then initiated by treatment with adenoviruses containing the pancreatic transcription factors Pdx1, MafA, Ngn3 and Pax4. Four days later (D4) the cells were treated with an siRNA to inhibit expression of the endogenous transcription factor ARX. The protocol was complete by D10. From D3-D10 the media was supplanted with Betacellulin, EGF, and nicotinamide.
Results: Immunocytochemistry revealed that the reprogrammed cell population was monohormonal, comprising 40% C-peptide-positive, 4% glucagon-positive, and <2% somatostatin-positive cells. The resultant Beta-like cells exhibited glucose responsive insulin secretion, expressed insulin protein levels (33.5 ± 7.3 pg/ ug protein) at around 15% of that in adult human islets, packaged insulin into secretory granules, and had an immediate and prolonged effect in normalising blood glucose levels upon transplantation into diabetic mice.
Conclusion: We describe here a highly reproducible and robust protocol that generates reprogrammed islet cells from the human exocrine pancreatic tissue that is normally discarded after the islet isolation procedure. The reprogrammed cells share many of the properties of adult endogenous Beta cells and compare well with surrogate Beta cells generated from human embryonic stem or iPS cells. We estimate that around 1.5 x 10^8 reprogrammed cells would have a therapeutic effect if transplanted in human diabetics; thus one recipient pancreas could provide numerous islet grafts.

Is-6: Progress towards An Allogeneic Bank of Stem Cell Derived Islets for Transplantation in The Treatment of Diabetes

Docherty K

School of Medical Sciences, University of Aberdeen, Aberdeen, UK
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Objective: The overarching aim of the study was to generate a replenishable supply of islets of Langerhans that would meet the ever-increasing demand for transplantation in the treatment of type 1 diabetes. There are two main alternative sources of islets for transplantation: ES/iPSC-derived progenitors, and fully transdifferentiated Beta cells derived from adult tissue. In the case of the latter the adult tissue could be expanded as a mesenchymal cell population (MSC), and then induced to undergo a reversal of the epithelial to mesenchymal transition (EMT) to provide a source of tissue that could be reprogrammed or transdifferentiated.
Materials and Methods: ES/iPS cells were differentiated into Beta-like cells following a protocol that recapitulates the pathway of development in the mouse, i.e. formation of definitive endoderm (DE), pancreatic progenitors, islet progenitors, and from there towards fully functional Beta cells. The protocol involves the sequential addition of the following factors: activin A to induce formation of DE, cyclopamine to induce foregut endoderm and mimic formation of the pancreatic anlage, retinoic acid and FGF to promote pancreatic morphogenesis, inhibition of activin signalling with SB431542 to block formation of liver lineages, Noggin to inhibit BMP signalling, and a gamma secretase inhibitor to inhibit exocrine and promote endocrine lineages. In the case of expanded adult tissue we have discovered that the EMT can be reversed by overexpressing KLF4.
Results: ES/iPS can be induced to differentiate towards Beta cell progenitors and functional Beta cells. The cells exhibit many of the properties of human adult Beta cells, including the ability to rescue diabetes in diabetic mouse models. Reversing the EMT that adult pancreatic tissue undergoes when placed in culture has proved more challenging. The amylase expressing acinar cells and residuals insulin expressing Beta cells can be genetically tagged with dsRED. The resulted AMY-Red and INS-Red MSCs were then expanded and FACs purified. Both populations could be differentiated into dsRED-labelled chondrocytes, osteocytes and adipocytes. We have further shown that KLF4 can induce a transient reversal of the EMT process, showing that both AMY-Red and INS-Red MSCs can be induced to express insulin, although at very low levels.

Conclusion: These results suggest that the goal of creating allogeneic banks of tissue typed Beta cells may be in sight. Avoiding the use of pluripotent cells, may have many translational advantages in terms of safety and complexity (cost/reproducibility) of the differentiation protocol.

Is-7: Non-Coding Genome Function in Pancreas Development and Disease

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Is-8: Use Epigenomics and Stem Cells to Identify A Role for Hippo in Early Pancreas Development

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Is-9: Induced Pluripotent Stem Cells for Disease Modeling

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Objective: Pluripotent stem cells provide a novel tool to study cardiomyogenesis as well as mechanisms of arrhythmias under control conditions as well as in disease models. We culti-vated embryonic stem (ES) and induced pluripotent stem (iPS) cells in 3-D cell aggregates (embryoid bodies, EBs), where they differentiate into derivatives of all three germ layers. Emphasis was put on the development of human ES based screening assays to predict toxic effects and to learn about toxicological mechanisms.
Materials and Methods: In order to demonstrate the suitability of pluripotent stem cells for novel disease models, reprogramming of fibroblasts from patients with LQT3, CPVT syndrome or chronic granulomatous disease (CGD) by ectopic expression of the transcription factors Oct4, Sox2, c-Myc and Klf4 resulted in generation of iPS cells for disease modelling.
Results: In order to offer several cell line models of LQT3, CPVT or CGD and therefore support research on pathophysiology and new therapeutic approaches, we optimized proto-cols to differentiate induced pluripotent stem cells (iPSCs) from wild-type, X0-, AR220- and AR470-CGD patient's fibroblasts into cardiomyocytes, neutrophils and into macrophages. Aberrant genetic clones were discarded after chromosome karyotyping and array-comparative genomic hybridization analysis.
Conclusion: We describe a reproducible, simple, and efficient way to generate cardio-myocytes, neutrophils and macrophages from iPSCs and provide a new cellular model for the AR220-CGD genetic form that has not been described before. This approach may also enable patient-specific cells which are an indispensable prerequisite for a later use in clinics as well as for personalized medicine.

Is-10: *In Vitro* and *In Vivo* Models for Cardiac Cell Therapy

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Objective: Functional integration and persistence of transplanted cardiomyocytes are crucial for a long-term therapeutic benefit of cardiac cell replacement therapy. Induced pluripotent stem cell-derived cardiomyocytes (iPSCM) are regarded as a promising cell type for cardiac cell replacement therapy, but long-term survival and functional integration of these cells have not been demonstrated yet. Thus, we investigated the long-term persistence, electrical integration and electrophysiological properties of transplanted iPSCM.
Materials and Methods: For lineage selection within the bioreactor and mass culture as well as to allow the identification of the transplanted cells, transgenic ES and iPS cells were used containing a vector with two cloning sites for EGFP and an puromycin resistance cassette for selection under the α-MHC promoter. We aimed at generating iPSCM and their molecular and functional characterization using genetic, molecular biological and physiological methods.
Results: To demonstrate the ability of pluripotent stem cells for regenerative medicine and tissue repair, CMs differentiated from iPS and ES cells were injected into the cryoinfarcted left ventricular wall of adult wild type mice. There is also growing interest in purified cardio-myocytes derived from embryonic stem cells for *in vitro* or *in vivo* tissue engineering. We established three-dimensional tissue culture model based cardiac slices, which were seeded either with highly purified cardiomyocytes that have been purified by puromycin selection, alone or in combination with purified fibroblasts. Long-term persistence and electrical integration of transplanted iPSCM into recipient hearts could be clearly demonstrated. Coupling of transplanted iPSCM and host tissue was already observed 6-12 days after transplantation. At later time-points, transplanted iPSCM were still located in recipient hearts and showed electrical coupling to host tissue. The collagen sponges that were transplanted with ES cell derived cardiomyocytes showed neither morphological nor functional in-

tegration of the cells. However, when co-cultured with embryonic fibroblasts cardiomyocytes formed fibre-like structures of rod-shaped cells with organized sarcomeric structure. As a consequence the engineered scaffold contracted spontaneously. Electrical coupling between cardiomyocytes was demonstrated by strong expression of connexin 43.

Conclusion: We conclude that fibroblasts are needed for morphological and functional engraftment of purified cardiomyocytes on collagen matrices. Translation from the laboratory into the clinic will be one of the future 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-11: Bioinformatics for Circulating Tumor Cell Genomics

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Is-12: miRNA Diagnostics for Minimally-Invasive Disease Detection

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Is-13: Advances in Islet Encapsulation for Transplantation

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Islet microencapsulation, where the islets are coated with a biocompatible polymer, has demonstrated various degrees of success in small and large animal trials. This success depends on many factors including alginate purity and the location at which the devices are transplanted. We evaluated alginate microcapsules made from two types of alginate hydrogels transplanted to one of two transplant sites into athymic nude mice to characterize changes in microcapsule morphology and foreign body and vascular response post-transplant.

Microcapsules generated with an air-driven, electrostatic microcapsule generator using either Sigma or ultra-pure low viscosity mannuronate (UPLVM) alginate solution at two concentrations (1.5 and 3%) were crosslinked in a gelling solution containing a 120 mM calcium chloride solution. The microcapsules were characterized using an inverted microscope after which they were transplanted at one of two sites in CD1 mice – dorsal subcutaneous space and the peritoneal cavity. The recipients were monitored for a 12 week period after which they were euthanized and the microcapsules were carefully collected for microscopic evaluation, dextran diffusion analysis, histological analysis and surface analysis.

After incubation at 37°C, alginate microcapsules (Sigma and UPLVM) showed a 14 ± 2% decrease in diameter. However, at explant, it was noted that the same microcapsules now showed a 31 ± 4% increase in diameter, which may be attributable to ionic interactions between sodium in the interstitial fluid and calcium in the capsules.

Microcapsules made from Sigma alginate elicited an intense inflammatory response and demonstrated macrophage infiltration in addition to dense fibroblast activation at both transplant sites. Microcapsules made with UPLVM alginate demonstrated a mild foreign body reaction with minimal peri-capsular fibroblast growth in the subcutaneous site while those transplanted in the peritoneal cavity were free floating and did not show any cellular infiltrate.

The results of this study suggest that the peritoneal cavity might be the ideal site for encapsulated islet and stem cell transplantation and UPLVM alginate being more biocompatible than Sigma alginate would be ideal for this purpose.

Is-14: Islet Transplantation Using Differentiated Human Stem Cells

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Diabetes is the 7th leading cause of death in the United States. Type 1 diabetes is an autoimmune disease where the body's immune system destroys the specialized insulin-secreting cells in the islets of Langerhans, called β cells. Insulin is the primary hormone that regulates blood glucose levels. In the absence of insulin, blood glucose levels will rise. Islet allotransplantation involves the transplantation of islets harvested from human pancreas donors into diabetic recipients and has the potential to help type 1 diabetics to improve autonomous regulation of their blood sugar levels. The biggest drawback is the need for long-term immunosuppression. Islet encapsulation is a technique that can protect the islets within biocompatible microscale devices before transplantation. Encapsulating islets within permeable hydrogels such as alginate provides an effective immune barrier and allows to transplant without immunosuppression.

In this study, we show that human embryonic stem cells derived-insulin-producing cells (hESC-IPCs) remain membrane viable and functional after alginate encapsulation and transplantation into diabetic rodents.
The hESC-IPC clusters were derived using a proprietary feeder-free, enzymatic propagation method. Using this method, a process that can take 12-13 weeks in the fetus can be completed in 18 days. The clusters were then shipped to UC Irvine where they were encapsulated in 2.5% (w/v) ultra-pure low viscosity high mannuronate alginate microcapsules using an air-pressure driven electrostatic bead generator (Nisco Engineering AG) using optimized settings (Voltage: 9 kV, Pressure: 3 psi, Needle height: 30 mm, Needle gauge: 25G, Agitator speed: 80 rpm, Cross-linking solution: 120 mM $CaCl_2$). After alginate encapsulation, the stem cells were allowed to recover overnight at 37°C, 5% CO_2 in RPMI supplemented with 10% human AB serum. The hESC-IPC clusters were then characterized and analyzed for membrane viability and function. The percentage of viable hESC-IPCs was analyzed using yo-pro-1, propidium iodide and calcein blue, quantified with a microplate reader (TECAN) and imaged using a fluorescence microscope (Nikon TIE).
Insulin release from hESC-IPCs was analyzed using a glucose-stimulated insulin response (GSIR) assay where known numbers of differentiated cell clusters were incubated for an hour each in the following solutions: low glucose (2.8 mM), high glucose (28 mM), high glucose + IBMX (28 mM +2 μM IBMX), and low glucose (2.8 mM). IBMX (3-isobutyl-1-methylxanthine) is an insulin secretagogue that further increases insulin release. The stimulation index (insulin release at high glucose concentrations /Insulin release at low glucose concentrations) and the maximum stimulation index (Insulin release at high glucose + IBMX/Insulin release at high glucose) was determined. Before encapsulation, the hESC-IPCs were 95.9 ± 0.02% viable and had a stimulation index (SI) of 0.57 ± 0.9. After alginate encapsulation, the viability was 93.9 ± 0.01% and the SI decreased to 0.25 ± 0.2. Statistical analysis demonstrated that alginate encapsulation did not negatively impact viability or function of the hESC-IPCs (P=0.38 and P=0.75 respectively). This indicates that the hESC-IPCs maintained viability and function after encapsulation. The hESC-IPCs maintained membrane viability and function after long-duration shipment and after encapsulation in alginate hydrogels. Studies that are currently underway include cellular characterization of hESC-IPCs using flow cytometry on free and alginate-encapsulated hESC-IPCs in order to evaluate the expression of insulin, glucagon and relevant stem cell markers in hESC-IPCs. This will be followed by *in vivo* studies where the encapsulated hESC-IPCs will be transplanted in diabetic mouse models to evaluate their efficacy.

Is-15: Bioinspired Substrates Direct The Fate of Stem Cells

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Objective: Bioinspired materials can mimic the stem cell environment and modulate stem cell differentiation and proliferation. In this context, biomaterials can mimic the biological microenvironments (i.e., niches) of stem cells and specifically affect the *in vitro* differentiation that is necessary for clinical application. *In vivo*, the appropriate differentiation, proliferation, and maintenance of potency are regulated by either stem cells or their specific niches. In this study, biomimetic micro/nanoenvironments were fabricated by cell-imprinted substrates based on mature human keratinocyte morphological templates and also smart nanoenvironments were obtained by cell-imprinted substrates based on mature and dedifferentiated chondrocytes as templates.
Materials and Methods :This substrate was characterized by SEM, AFM, Flouresent and Confocal microscopy. Toxicity of PDMS to stem cells was evaluated using an MTT-assay. The gene expression analysis of differentiated cells, were detected by Real Time PCR, array analysis and computer simulation study.
Results: The data obtained from atomic force microscopy and field emission scanning electron microscopy revealed that the keratinocyte-cell-imprinted poly (dimethylsiloxane) casting procedure could imitate the surface morphology of the plasma membrane, ranging from the nanoscale to the macroscale, which may provide the required topographical cell fingerprints to induce differentiation. Gene expression levels of the genes analyzed (involucrin, collagen type I, and keratin 10) together with protein expression data showed that human adipose-derived stem cells (ADSCs) seeded on these cell-imprinted substrates were driven to adopt the specific shape and characteristics of keratinocytes. The observed morphology of the ADSCs grown on the keratinocyte casts was noticeably different from that of stem cells cultivated on the stem-cell-imprinted substrates. Since the shape and geometry of the nucleus could potentially alter the gene expression, we used molecular dynamics to probe the effect of the confining geometry on the chain arrangement of simulated chromatin fibers in the nuclei. Additionally, rabbit adipose derived mesenchymal stem cells (ADSCs) seeded on these cell-imprinted substrates were driven to adopt the specific shape (as determined in terms of cell morphology) and molecular characteristics (as determined in terms of gene expression) of the cell types which had been used as template for the cell-imprinting.
Conclusion: The results obtained suggested that induction of mature cell shapes onto stem cells can influence nucleus deformation of the stem cells followed by regu-

lation of target genes. This might pave the way for a reliable, efficient, and cheap approach of controlling stem cell differentiation toward human cells for wound healing applications.

Keywords: Stem Cells, Biomimicking, Cell Fate, Differentiation, Smart Substrates

Is-16: Mouse Bone Marrow Mesenchymal Stem Cells, as A Model for Evaluating Nanomaterial Toxicity

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Mesenchymal stem cells are multi potent progenitor stem cells that have the ability to differentiate into atleast three lineages such as chondrocytes, osteoblasts and adipocytes and are one of the most widely used cells in tissue engineering. Owing to high proliferative and differentiation capacity, Mesenchymal Stem cell culture could be considered as a model of target tissue in the body and thereby mimics the tissue response to the exposure of nanomaterials. For these reasons, stem cells could be exploited for developing *in vitro* model systems to evaluate the toxicity of nanomaterials.

The objective of the study is to investigate the mouse bone marrow mesenchymal stem cells (BMSCs) as a model for evaluating the nanomaterial toxicity.

Stem cells isolated from the bone marrow of Swiss albino mice (BMSCs) were used for the study. The BMSC were cultured and maintained in DMEM-HG medium and are further characterized for the expression of MSCs surface markers CD44, CD90 and negative marker CD45. Confluent cells obtained after third passage were used for the toxicity evaluation. Different concentrations of HANPs (hydroxyapatite nanoparticles) and ZONPs (zinc oxide nanoparticles) were exposed to BMSCs. Various toxic parameters such as cytotoxicity, reactive oxygen species (ROS) production and apoptosis by Annexin V/PI assay using FACS were evaluated.

The results of the MTT assay indicated that HANPs does not induce cytotoxicity up to 800 µg/mL. It was also observed that oxidative stress related apoptosis and ROS production following HANPs treatment was similar to that of control. Further, ZnO NPs significantly affects cellular viability in a dose dependent manner. Formation of ROS was found to be the mechanism of cellular toxicity. The release of Zn+ ions from the nanoparticles, due to the instability of ZnO NPs in the acidic compartment of lysosomes also increases the ROS generation. In addition to increased ROS production, damage of lysosomal membrane and the activation of executioner caspase-3 and caspase-7 were observed which eventually ends in apoptosis.

The present study concludes that the in house synthesized HANPs are non-toxic/safe at the molecular level suggesting that the HANPs are compatible to BMSCs. However, a dose dependent toxicological response of ZnONPs to bone marrow mesenchymal stem cells are observed as evidenced from the potential to induce cytotoxicity and ROS generation which in turn affects the cytoskeleton organization and disrupts the intracellular interaction. Activation of caspases and flow cytometry data reveals oxidative stress mediated apoptosis. Furthermore the toxicity mechanism of ZnONP interaction with BMSC elucidated in the present study followed both the mitochondria and lysosome mediated programmed cell death. Based on the parameters evaluated, it is concluded that BMSCs could be used as an alternative model for evaluating the preliminary toxicity of nanomaterials.

Is-17: Rabbit Adipose Derived Mesenchymal Stem Cells for Bone Interface Regeneration

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Adipose derived Mesenchymal Stem Cells (ADMSCs) have become a focus of research due to their multipotential properties. Adipose tissue is abundant in both humans and animals and can be easily harvested from subcutaneous tissue through percutaneous or limited open aspiration techniques and provides large volume of viable pluripotent stem cells. Adipose is the most profuse source of adult stem cells, and thus it holds great promise for use in tissue repair and regeneration and it shows fibroblastic morphology and characteristics.

The objective of the present study is to evaluate the compatibility of tissue-engineered Hydroxyapatite-Burr Hole Button device (HAP-BHB) seeded with ADMSCs for bone regeneration.

Burr-hole cranial neurosurgical procedures lead to the development of cosmetically puckered scars on the scalp over burr-hole sites. Ceramics, especially hydroxyapatite (HA) are good bone substitutes owing to their biocompatibility and osteo-conduction. The ADMSCs were cultured and maintained in DMEM-HG medium and are further characterized for the expression of MSCs surface markers CD44, CD90 and negative marker CD45. Confluent cells obtained after third passage were used for the study. Here, ADMSCs were seeded on to HAP-BHB and the cytotoxicity, oxidative stress response, apoptotic behavior, attachment, adherence were evaluated to predict efficacy of using ADMSC seeded Hydroxyapatite-Burr Hole Button device for bone interface regeneration

The results of the 3-(4, 5- MTT dimethylthiazol)-2,5-diphenyl tetrazolium bromide) assay indicated that pow dered device material was non-cytotoxic up to (0.5 g/ml) on cultured cells. It was also observed that oxida-

tive stress related reactive oxygen species (ROS) production and apoptosis on cell seeded device was similar to that of control (cells alone) except in 3 days period, which showed an increased ROS generation. Scanning Electron and Confocal microscopy indicated a uniform attachment of cells and viability up to 200 nm deep inside the device.
Based on the results, it can be concluded that the in-house developed HAP-BHB device seeded with ADMSCs are non-toxic, compatible device for biomedical application and an attractive tissue engineered device for calvarial or similar defect regeneration.

Is-18: The Origin of The Brain

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Basic studies in model systems such as the frog and the mouse have been instrumental for our current understanding of evolutionarily conserved principles of organogenesis and development. During corticogenesis, however, there are features unique to primates that are not possible to model in non-primate systems. Since human embryonic stem cells (hESCs) have the capacity to give rise to all cortical neuronal derivatives in an intrinsic manner, hESCs provide a window into developmental events in the human embryo. By combining human genome modification technologies with the "default model" of neural induction, we are now able to probe fundamental questions of human corticogenesis. Using these genetic tools, we have demonstrated that hESC derived neuroepithelium recapitulates most aspects of their mammalian counterparts *in vivo* and have begun to uncover novel mechanisms for generation of the various cortical layers involved in thought, abstraction, and human cognition. Our paradigm allows us to make predictions about human corticogenesis that we are testing in fetal samples.

Is-19: Proteomic Analysis of A Stem Cell Model of Pediatric Motor Neuron Disease

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Is-20: The Ethics of How Stem Cell Research Is Represented

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Is-21: Mechanical Stimulation as Anabolic and Anti-Catabolic Agents in Bone Tissue Engineering

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Objective: In clinical situations, bone defects are often located at load bearing sites and hence bone substitutes or scaffolds are subjected to mechanical stimulation. Thus, it is critical to understand the effect of mechanical stimulation on bone formation inside bioresorbable bone scaffold. In fracture healing, the loading effect has been often reported to enhance the bone healing process. However, little is known on the effect of loading on bone formation inside scaffold. The goal of this study is to test the hypothesis that cyclic loading enhances bone formation inside a polymeric bioresobable bone scaffold.
Materials and Methods: Both femoral condyles of 8 female Wistar rats of weight 245-250 g were drilled (Veterinary Authority from the Canton of Vaud, authorizations No. 2140) and PLA + 5% wt β-TCP scaffolds of the same size were implanted inside the drilled holes. Prior to surgery, scaffolds were perfused with PBS to remove the air bubbles trapped inside. No cells or growth factors were added in the scaffold. Three days after surgery, the right knee of all rats were loaded and the left knee was used as control. A compressive load of 10 N at 4 Hz for 5 minutes was applied by a compression machine 5 times every other day. Both knees were micro-scanned *in vivo* (SkyScan 1076, Belgium) at 2, 4, 6, 9 and 13 weeks after surgery. For the scanning, the knee was extended. The CT parameters were: 18 μm resolution, 80 kV voltage, 124 μA current, 1mm Al filter and 600 ms exposure time. Reconstruction and analysis were done using NRecon and CTan software (SkyScan, Belgium), respectively. Bone mineral density (BMD) was calibrated using two phantoms. BMD of 0.5 g/cm^3 was chosen to segment bone. Accordingly, bone volume (BV) and BMD of bone inside scaffold were measured.
Results: Already at 2 weeks, small amount of bone formation can be seen inside the scaffold, which was mainly formed at the outermost part of the scaffold. At 13 weeks, new bone invaded the interior of scaffold and was well spread. For BV, both intercept and slope of equation 1 were highly significantly different (P value < 0.0001) for control and loaded groups. This means that loading had a negative effect at the beginning, but a positive effect later. The bone formation rate inside the scaffold was indeed increased by 28%, which after 13 weeks resulted in more bone formation in the loaded group.
Conclusion: This study confirmed the hypothesis that mechanical stimulation enhances the bone formation

inside a resorbable bone scaffold. We also observed that a short stimulation period after surgery resulted in a long-term increase of bone volume. The observed early negative effect of mechanical stimulation might be due to high strains at the bone-scaffold interface. This adverse effect could be avoided by a gradual increase of the load. Nevertheless, this negative effect was cancelled after 6 weeks, and at 13 weeks the bone volume was significantly higher in the loaded group. In conclusion, mechanical stimulation can be a potent stimulatory signal signal for bone Formation within bioresobable bone scaffolds.

Is-22: Photoreceptor Transplantation for The Restoration of Daylight Visual Responses in A Mouse Model of Cone Degeneration

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Objective: Visual impairment and blindness due to the loss of photoreceptors represent one of the main causes for disability in industrialized societies. Currently no established therapy is available to rescue lost visual function. Cell replacement strategies by means of transplantation represent promising treatment approaches. Indeed, in recent years methods were developed to isolate, enrich and inject photoreceptors into animal models of retinal degeneration. However, the majority of photoreceptor transplantation studies so far used the mouse as a model system, thereby focusing on rod photoreceptors. Rods are functional in dim light conditions and therefore the nocturnal mouse retina is rod dominated. In contrast, human vision mainly depends on daylight vision provided by cone photoreceptors that also allow color detection. Here, we evaluated the potential of photoreceptor transplantation for daylight vision repair.
Materials and Methods: As the mouse retina is a poor source for cones, we took advantage of cone-only retinas of neural retina leucine zipper-deficient (Nrl $^{-/-}$) mice as a comprehensive source for transplantation studies. GFP-labeled Nrl $^{-/-}$ cone-like photoreceptors were enriched by CD73-based magnetic activated cell sorting and transplanted to the sub-retinal space of wild-type mice and a mouse model of cone degeneration, the cone photoreceptor function loss 1 (Cpfl1) mouse. 2, 4, 12 and 24 weeks post-transplantation experimental animals were investigated by immunohistochemistry, electron-microscopy and micro-electrode arrays (MEA).
Results: Donor cone-like photoreceptors integrated into the outer nuclear layer of host wild-type and Cpfl1 retinas and generated a mature photoreceptor morphology including synapses and outer segments. Donor photoreceptors expressed cone-specific markers including s-opsin, cone arrestin and peanut agglutinin. Analysis by MEA showed stimulus-driven ON, OFF and ON-OFF responses in retinal ganglion cells of Cpfl1 hosts under intensive light conditions.
Conclusion: The transplantation of cone-like photoreceptors provided the proof-of-concept for the feasibility of daylight vision restoration upon cell transplantation in the mammalian retina.

Is-23: Heart, Liver and Pancreas Regeneration: Common Themes?

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My lab investigates questions related to organogenesis including cell differentiation, tissue morphogenesis, organ homeostasis and function, as well as organ regeneration. We study these questions in zebrafish as well as in mouse and are currently looking at several mesodermal (heart, vasculature) and endodermal (pancreas, lung, liver) organs. We utilize both forward and reverse genetic approaches, and aim to dissect cellular processes using high-resolution live imaging. One goal of our studies is to gain understanding of vertebrate organ development at the single-cell level, and beyond. This talk will focus on small molecule screens relevant to glucose homeostasis, including looking for compounds that increase pancreatic beta-cell regeneration.

Is-24: Imaging Heart Formation and Function in Zebrafish

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This talk will focus on cardiac development and function.

Is-25: Establishing and Maintaining Stem Cells

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Objective: Distinct gene regulatory networks operate in different anatomical locations to give rise to skeletal muscle stem and progenitor cells. One question is the requirement for 3 cell fate determinants, Myf5, Myod and Mrf4, to establish muscle cell fate. Another is how stem cells are maintained throughout tissuegenesis.
Materials and Methods: Genetic lineage tracing studies combined with cell ablations and conditional expression of Notch pathway are employed in genetically

modified mice to examine *in vivo* relationships of stem and differentiated cells.
Results: Was gene duplication of critical cell fate determinants during evolution associated with duplication of the lineage to introduce a failsafe mechanism in case of tissue failure, or do these determinants act within a single lineage? We performed cell ablation studies using combinations of mouse mutants to address this question. We also show the cell fate determinants act in one progenitor cell lineage in contrast to a prevailing model proposed. We show also that prenatal muscle stem cells are maintained by constitutive expression of Notch.
Conclusion: Our findings lead us to propose that the myogenic cell fate determinants act largely in a single progenitor population and therefore distinct lineages are not contributing to the heterogeneity of the adult stem cell population. Furthermore, Notch signaling is compatible with muscle stem cell maintenance and proliferation, as well as maintaining the adult quiescent muscle stem cell state.

Is-26: Generating Muscle Stem Cells and Assessing Heterogeneities for Regenerative Medicine

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Objective: Stem cell maintenance requires dialogue between the stem cell and its niche. The factors that are required for determining stem cell properties and divisions were investigated using artificial niches.
Materials and Methods: Micropattern technologies were used to design artificial niches for propagating stem cells and investigating their modes of division: symmetric and asymmetric. This was combined with genetically modified mice and induced skeletal muscle regeneration.
Results: Two types of asymmetry were followed to investigate stem cell properties: asymmetric DNA segregation (non-random DNA segregation) and asymmetric segregation of transcription factors (Pax7, stem; Myogenin, differentiated). By studying single stem cell divisions on micropatterns in artificial niches, we show that altering the topology of the niche impacts of the type of division that the stem cell will perform.
Conclusion: Using micropattern technology and single cell analysis, we show that extrinsic adhesion cues play a major role in determining cell fate outcomes of muscle stem cells.

Is-27: Recapitulation of Early Embryonic Spatial Patterning in Human Embryonic Stem Cells Using Micropatterned Control of Colony Architecture

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Embryos allocate cells to the three germ layers in a spatially ordered sequence. While in response to growth factors, human embryonic stem cells (hESCs) can generate the three germ layers in culture, differentiation is typically heterogeneous and spatially disordered. Here we show using micropatterning technology that geometric confinement to circular disks similar in size to mammalian embryos is sufficient to trigger self-organized patterning in hESCs. In response to BMP4, these colonies differentiate to an outer trophectoderm-like ring, an innerectodermal circle and a ring of mesendoderm in between, in an ordered, reproducible sequence along the radial axis of the colony. Fates are defined relative to the boundary, but have intrinsically determined dimensions: small colonies correspond to the outer layers of larger ones. Self-organized cell communication limits the range of BMP4 signaling to the colony edge but induces a broader gradient of Activin/Nodal signaling that patterns mesendodermal fates. These results demonstrate that the intrinsic tendency of stem cells to make patterns can be harnessed by controlling colony geometries, and provide a quantitative assay for studying paracrine signaling in hESCs.

Is-28: Base Excision Repair in Epigenetic Regulation of Mammalian Development

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DNA transactions including base modification and repair function to maintain the genome integrity and ensure proper gene expression. One of the known mammalian glycosylases, thymidine DNA glycosylase (TDG) is able to recognize G/T mispair and excise the thymine specifically to initiate base excision repair (BER). However, knockout of the TDG gene in mouse doesn't increase genome instability but instead leads to mis-regulation of gene expression and embryonic lethality. This observation has suggested a role of base excision repair in epigenetic regulation. In our recent work, we demonstrate that TDG recognizes and excises the 5-carboxylcytosine, an oxidation product of 5-methylcytosine generated by the Ten-Eleven-Translocation (Tet) family of dioxygenases. In mouse embryonic stem cells, ablation of TDG blocked the demethylation process mediated by Tet enzymes. Mouse embryonic fibroblasts (MEFs) deficient in TDG were unable to be reprogrammed into induced pluripotent cells (iPSCs). We conclude that TDG

together with Tet mediate DNA demethylation for the erasure of epigenetic barrier in development as well as in cell reprogramming.

Is-29: DNA Oxidation towards Totipotency in Mammalian Development

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Objective: Mammalian development begins with a single cell resulted from the fertilization of a sperm and an oocyte. The early embryonic genome undergoes profound epigenetic reprogramming to prepare for development. We try to understand the biological significance and mechanisms of epigenetic reprogramming.
Materials and Methods: We used biochemical assays to detect oxidation product of 5-methylcytosine in DNA and identify the responsible oxidases. The significance of 5mC oxidation was confirmed by mouse gene targeting experiments.
Results: We find that 5-methylcytosine (5mC), the most abundant type of base modification in DNA, is oxidized to 5-hydroxymethylcytosine (5hmC) as well as 5-carboxymethylcytosine (5caC) in mouse zygotes. *In vitro*, the Tet family of dioxygenases oxidize 5mC to 5caC under physiologically relevant conditions (e.g. in the presence of 1mM ATP). In zygotes, the Ten-eleven-translocation protein Tet3 is responsible for the genome-wide oxidation of 5mC to 5hmC and 5caC. Deficiency of zygotic Tet3 impedes demethylation at the paternal Oct4 and Nanog genes and delays the reactivation of Oct4 in early embryos. The heterozygous mutant embryos lacking maternal Tet3 suffer increased developmental failures. Importantly, oocytes lacking Tet3 also show impaired reprogramming of injected somatic cell nuclei. In addition, MEFs deficient in all Tet genes were unable to be reprogrammed by Yamanaka factors.
Conclusion: We conclude that Tet-mediated oxidation is important for DNA demethylation and gene activation in the early embryo following natural fertilization, as well as for the reprogramming in somatic cell nuclear transfer and factor-based iPSC generation.
Keywords: Epigenetic Reprogramming, DNA Demethylation, Enzymatic DNA Oxidation, Cell Pluripotency

Os-1: Allogeneic Stem Cell Transplantation for Myelofibrosis in The JAK1/JAK2 Inhibitor Era

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Objective: Allocating patients with myelofibrosis (MF) to allogeneic hematopoietic cell transplantation (HCT) is complex. Patient-, MF-, and transplantation-related aspects need to be considered for decision making The JAK1 and JAK2 inhibitor ruxolitinib is effective in decreasing symptomatic splenomegaly, and MF-related symptoms. However, HCT remains the only curative option. We evaluated the impact of ruxolitinib treatment prior to HCT on outcome after HCT.
Materials and Methods: A cohort of 14 patients with MF (median age 58 years) were treated with the JAK1/JAK2 inhibitor ruxolitinib and received subsequently HCT from related (n=3), and unrelated (n=11) donors after a median exposure to ruxolitinib of 6.5 months. At HCT, MF-risk for survival according to the International Prognostic Scoring System was intermediate-2 or high risk in 86% of patients.
Results: Under ruxolitinib, MF-related symptoms were ameliorated in 10 (71.4%) patients and the palpable spleen reduced by a median of 41% in 7 (64%) of 11 patients with splenomegaly. Engraftment occurred in 13 (93%) patients. Acute graft versus host disease (GvHD) grade-III occurred in 2 (14%) patients. Survival, event-free survival, and treatment-related mortality were 78.6, 64, and 7%, respectively.
Conclusion: Through the reduction in both cytokines and splenomegaly with ruxolitinib as well as improvement in performance status, ruxolitinib might improve outcome after allogeneic HCT in patients with MF. The down-regulation of inflammatory cytokines might have a beneficial impact on graft failure and acute GVHD. potential risks assosiated with the use of JAK1/JAK2 inhibitors in the transplant setting such as cytokine rebound and the impact of immunmodulation on infections need to be carefully studied.
Keywords: JAK Inhibitor, Ruxolitinib, Myelofibrosis, Allogeneic Hematopoietic Cell Ttransplantation

Os-2: Alginate Composition and Temperature Influence Microcapsule Permeability

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Objective: Alginate encapsulated islets have the potential to cure Type I diabetes in humans and provide long-term protection from immune-mediated graft damage. Alginate pore size is a crucial parameter that aids in the protection of islets from host immune recognition, while allowing oxygen and insulin to diffuse into the capsules. The aim of this study is to determine the pore size of alginate microcapsules using dextrans of specific sizes to identify capsules with optimal diffusion parameters.
Materials and Methods: Alginate microcapsules were made with 2.5% (w/v) ultra-pure low viscosity high mannuronate (UP LVM) and ultra-pure low viscosity high guluronate (UP LVG, NovaMatrix® PRONOVA™) using a microcapsule generator (Nisco Engineering AG) at standard settings (9 kV; voltage, 80rpm; stirrer speed, 3psi; air pressure, 30 mm; needle height, 25 G; needle gauge, 120 mM $CaCl_2$; gelling solution). The microcapsules were then incubated at either 3°C or 37°C for a 24 hour period. Samples were taken post-incubation and plated on a slide flask to which a cocktail of three fluorescently-tagged dextrans were added: 3mg/mL of cascade blue conjugated 10kDa (Life Technologies, cat. D-1976), 15 mg/mL of fluorescein isothyocyanate (FITC) conjugated 150 kDa (Sigma-Aldridge, cat. FD150S-1G), and 15mg/mL of tetramethylrhodamine isothiocyanate conjugated 500 kDa (Sigma-Aldridge, cat. 52194-1G). Images were obtained using a two-photon confocal microscope (Zeiss LSM 520 Meta, LSM 4.2 SPI software by Carl Zeiss) at 0 and 30 minutes. The images were analyzed using the image analysis software, ImageJ, to determine the percentage change in fluorescence intensity inside the capsules. All data is reported as Mean ± SEM. Statistical analysis was performed using a one way ANOVA and $P<0.05$ was considered statistically significant.
Results: 10kDa dextrans were able to freely permeate both UP LVM and UP LVG alginate microcapsules with no significant change from 0 to 30 minutes at 3oC and 37oC. Thus, a small molecule like insulin (5-6 kDa) would be able to freely diffuse out of these microcapsules. 500kDa dextrans showed significantly higher diffusion in UP LVG (26.26 ± 0.9%; 3 °C, 16.25 ± 1.1%; 37°C) than UP LVM (0%; 3°C, 0%; 37°C), indicating that UP LVM alginate microcapsules are relatively impermeable to very large protein molecules. UP LVG microcapsules demonstrated significantly higher diffusion of 150 kDa dextrans (72.3 ± 0.7%; 37°C, 73.3 ± 0.5%; 37°C) when compared to UP LVM microcapsules (32.0 ± 1.1%, 3°C, -4.6 ± 0.5%; 37°C). This indicates that microcapsules show rapid diffusion of 150kDa dextrans independent of incubation temperature while high M alginate microcapsules do not ($P<0.001$, ANOVA). Interestingly, a temperature-dependent reduction in dextran diffusion (and hence alginate pore size) is noted in UP LVM alginate microcapsules, a property that would enhance exclusion of IgG (~150 kDa) and other antibodies after transplantation.

Conclusion: The results of this study suggest that alginate composition (specifically, guluronic acid content) and temperature greatly influence pore size and permeability. These results suggest that high mannuronate alginate microcapsules may confer significantly better protection from the humoral immune system (IgG antibodies); they are preferred candidates for further *in vivo* encapsulated islet transplant studies in small and large animal models.

***Keywords*:** Alginate, Immune Response, Permeability, Transplant

Os-3: Epigenetic Mechanisms in Neural Stem Cells; Implicating The Role of DNA Methylation and MeCP2

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Multipotent neural stem cells (NSC) self-renew and differentiate into different cell types of the central nervous system. The process of NSC differentiation is tightly controlled by epigenetic mechanisms that dictate NSC fate commitments. An important epigenetic modification with key roles in brain development and NSC differentiation is methylation of DNA molecules. Two most studied types of DNA methylation are 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). My lab studies the role of 5mC and 5hmC in NSC differentiation and their impact in neurological disorders. Our studies are mainly focused on MeCP2, which is the main 5mC- and 5hmC-binding protein in brain. MeCP2 is a highly abundant epigenetic factor in brain and its loss-of-function mutations cause Rett Syndrome, a severe from of autism spectrum disorders. Currently, Rett Syndrome has no cure and the pathobiology of the disease is not fully understood.

Our initial studies in MeCP2-deficient mouse were proof-of-principle for the role of MeCP2 isoforms in differentiated adult NSC. We reported the first generation of preclinical MeCP2 gene therapy vectors and performed high efficient gene therapy delivery into NSC, neurons and brain microenvironment. Since 2009, my lab has devoted an intensive focus on the transcriptional control and functional role of MeCP2 isoforms and MeCP2 genetic-epigenetic regulatory networks in neural stem cells. This knowledge is critical to establish outcome measures in identifying possible therapy strategies for Rett Syndrome. To this end, we recently identified an FDA approved drug (Decitabine) that induces MeCP2 expression in neural stem cells via altered DNA methylation at the cis regulatory elements. In differentiating NSC, we showed that Decitabine globally alters 5mC and 5hmC levels. In a side-by-side comparison of Decitabine and selected chemical compounds (proposed for Rett Syndrome therapeutic applications), we studied their impact in activating MeCP2 regulatory network and promoting NSC neurogenesis. Our data are not only important for therapeutic applications of Rett Syndrome, but also for other MeCP2-associated neurological conditions, such as autism, and X-linked mental disability, that currently have no cure.

Ps-1: A Modified Model of Primary Cilium to Investigate its Response to Fluid Flow

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Objective: The primary cilium is an organelle which occurs singly on nearly every cell in the vertebrate body. They are considered important in the osteogenic lineage specification of mesenchymal stem cells (MSC) and human adipose-derived stem cells (hASC). In this study, we simulated the primary cilium using a modified model to derive the experienced strain and stress, which are determining factors in function of stretch-activated ion channels. Located in the membrane of the cilium base, these channels play a pivotal role in many cilia-related reactions and diseases.
Materials and Methods: Primary cilia, organelles which comprise a growing area of investigation in bone tissue engineering, are being studied using a new model in this piece of research. We thought of primary cilium as a beam which is attached to the cell through an ideal torsion spring. This model is then submerged in the fluid, which exerts the driving shear force, and is subsequently analyzed using finite element methods and fluid-structure interaction techniques.
Results: The analysis is carried out using COMSOL multiphysics. The model is a two dimensional strip which is bound to the cell by means of a thin layer with adjustable spring constant, while it is free to bend in response to the fluid flow. As a result, cilium experiences considerable stress at the base, which is why scientists think of it as a cellular mechanosensor. We have seen that applying such a boundary condition decreases stress by 40% in our approximate model.
Conclusion: In previous studies the primary cilium was mathematically modeled using a cantilevered beam which was at a fixed angle with respect to the cell. This assumption, however, leads to an oversimplified model which in turn, gives rise to misleading results. We have shown that inclusion of a torsion spring dramatically reduces the maximum stress experienced by the cilium.
Keywords: Primary Cilium, Mechanosensation, Mechanical Stimuli, Fluid-Structure Interaction, Finite Element Method

Ps-2: *In Vitro* Isolation, Culture and Identification of Human Endometrial Mesenchymal Stem/Stromal Cells (EnMSCs)

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Objective: Dynamic remodeling of endometrium in each menstrual cycle (regeneration, differentiation, and shedding), for the purpose of its embryo implantation preparing, may be a reason on ability of endometrial basal layer cells for proliferation and differentiation into different cell types, that these functions are highly regulated by the stem cells. It has been hypothesized that adult stem/stromal cells are responsible for the cyclical regeneration during a woman's reproductive phase. Medical application of stem cells could be for uterine-factor infertility which is caused by absence or dysfunction of the uterus in the future.
Materials and Methods: Human endometrial tissues were collected from 3 cycling women after fully consent. Human endometrial tissues were scraped and dissociated into single-cell suspensions with mechanical and enzymatic digestion, prepared for cell culture. Cells were cultured in DMEM containing 10% FBS and incubated at 37ºC in 5% CO_2. Endometrial mesenchymal stem/stromal cells (EnMSCs) were characterized by immuno-localisation with CD34, CD44, CD105, VIMENTIN, TRA-1-81, and TRA-2-54 markers.
Results: EnMSCs were isolated, propagated (passaged more than seven times), frozen, thawed, and identified with specific marker for mesenchymal stem cell (CD105), hematopoietic stem cell marker (CD34), and mesenchymal stromal cell marker (VIMENTIN). However, adhesion marker (CD44), and human embryonic stem cell markers (TRA-1-81 and TRA-2-54) have not been expressed. Moreover, these cells were morphologically similar to mesenchymal stem cells (MSCs) in culture.
Conclusion: Our preliminary data confirms other findings regarding isolation, culture and characterization of human EnMSCs. The endometrial tissue is an available source of MSCs acquirable with minimum side-effects that could be used for the future clinical applications for the patients with various types of uterine dysfunctions which can leads to recurrent pregnancy loss and finally resolve of surrogacy problems.
Keywords: Endometrial Mesenchymal Stem/Stromal Cells, Endometrium, Mesenchymal Stem Cells

Ps-3: Comparison of Human Osteoblast Proliferation in Alginate and Hydroxyapatite-Tricalcium Phosphate Scaffolds

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Objective: After bone fractures from traffic trauma, many patients suffer from non-healing bone defects. So, it is important to identify modern and effective methods to improve healing of bone defects. One of them is, using bone cells from the patient himself, culture of these cells on appropriate scaffold and finally, transfer them to injured area.The main objective of this study is to compare the rate of osteoblast proliferation in alginate beads, and hydroxyapatite-tricalcium phosphate (HA-TC) scaffold.
Materials and Methods: Bone tissue specimens were obtained from 4 patients undergoing craniotomy surgery. Bone specimens were cut in to small pieces and put in Petri dishes having culture medium. The cell cultures reached confluence, averagely after 2 weeks time. First passage cells were divided in two portions. One portion was used for hydroxyapatite-tricalcium phosphate scaffold, and the other was added to alginate gel. After a 2 weeks period, the data were collected and analyzed.
Results: The osteoblasts in hydroxyapatite-tricalcium phosphate scaffold and alginate gel, had round morphology. Van kossa staining demonstrate, mineralized matrix in both groups. The number of harvested cells in 2 weeks after culture were significantly higher in Alginate group ($P<0.001$). In addition MTT assay shows significant difference in the mean of viability rates between both groups in day 14 ($P<0.001$).
Conclusion: This study showed that Alginate gel support better proliferation and viability of osteoblasts in comparison with the hydroxyapatite-tricalcium phosphate scaffold. The probable cause of these differences can be searched in Alginate bio properties; porosity of Alginate gell provide conditions in which cellular and metabolic activities have been accelerated.
Keywords: Alginate, Bone healing, Hydroxyapatite-Calcium Phosphate, Osteoblast

Ps-4: Ly294002 Small Molecule Promote The Expression of Motor Neuron Genes

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Objective: Phosphatidyl inositol 3-kinase (PI3K) is an enzyme implicated in signal transduction by associating with receptor and nonreceptor tyrosine kinases. PI3K/Akt signaling has been implicated in multiple cellular and organ functions, including growth, cell survival, glucose metabolism and protein synthesis. Reports have shown that during neural differentiation, PI3K/Akt over-expression reduces glial (GFAP) and neuronal (b-tubulin III) marker expression and inhibits cell differentiation. It has been found that Ly294002 small molecule can inhibit PI3K/Akt pathway and promote neural differentiation. The human endometrial stem cells (hEnSCs) are capable of extensive self-renewal and studies indicate that these cells have chondrogenic, osteogenic and neurogenic potential. The purpose of this study was to investigate the effect of Ly294002 small molecule on the expression of motor neuron genes.
Materials and Methods: Endometrial biopsies were obtained from patients and then the cells were extracted enzymatically and cultured in DMEM/F12 medium supplemented by 10% FBS. The flow cytometry analysis was done for haemopoietic marker (CD34), mesenchymal stem cell markers (CD146, CD90 and CD105) and endothelial marker (CD31) in the third passage. In the first step of cell differentiation, FGF2, IBMX, B27 and 2ME was added to the media for one day. Then cells were treated with N2/B27 and Ly294002. After 3 days, Retinoic acid (RA) and Ly294002 was added to the media for 8 days. At the last, cells were treated with N2/B27 and BDNF for other 7 days. The mRNA levels of several genes were measured including NF, Nestin, chat and GFAP by Real-time PCR Technique.
Results: Real-time PCR data showed that mRNA levels of NF, Nestin, chat and GFAP were increased in LY294002 treated hEnSCs.
Conclusion: Our data suggest that Ly294002 can promote the neuronal fate decision in hEnSCs through promoting the expression of motor neuron genes and inhibition of the PI3K/AKT pathway.
Keywords: HEnSCs, Motor Neuron, Ly294002, Phosphatidyl Inositol 3-Kinase (PI3K)

Ps-5: A Useful Method of Platelet Rich Plasma (PRP) Isolation and Preparation in Human

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Objective: To date, platelet-rich plasma (PRP) is widely applied in medical treatment as a pool of different growth factors such as PDGF, IGF, TGF, BGGF, and BDNF. This worthy component is used for improving tissue regeneration in healing therapies, plastic surgery and orthopedics. So, we reported an affected PRP isolation protocol to standardize a methodology for autologous platelet rich plasma in human.
Materials and Methods: 15 ml peripheral blood from six healthy donors (male- between 20- 30 years old) was collected. Mechanical procedures such as fast pipetting, strong shaking can activate platelets and should

be noticed. At first, freshly collected blood was centrifuged for 5 min at 280×g and RT to reduce the number of red and white blood cells. At second, centrifugation was done at 450×g for 12 minutes at RT to settle platelets. PRP samples were counted by the Neubauer chamber compared to peripheral blood platelets as a control group.
Results: We recovered nearly 4 to 6 folds increase in platelet concentration (1.2×10^6 to 1.9×10^6 platelets/µl). The concentration of red/ white blood cells was only <1% at final PRP preparation.
Conclusion: PRP has been recently considered as a growth factor source in different clinical therapies. One of the most negatively important factors at this component is blood-derived cell types that should be eliminated from PRP because of its potential proinflammatory effect. Reduced concentration of leukocyte is one the advantages of this protocol. Less time to perform platelet isolation can also improve the PRP preparation procedure.
Keywords: Platelet Rich Plasma, Leukocyte, Growth Factor, Platelet Count

Ps-6: Wnt5A Promotes Spheroid Formation and Increase Integrinb1 Expression in Human Ovarian Cancer Cell Line; SKOV-3

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Objective: Metastasis of ovarian cancer (OC) requires formation of OC cell spheroids in peritoneum and their further adherence to mesothelium which is rich in collagens, laminin and fibronectin and requires integrinb1 up-regulation in OC cells. Our previous study showed that Wnt5A influence adhesion and survival of monolayer culture of human OC cell line, SKOV-3. This study sought to determine Wnt5A role in spheroid formation of SKOV-3 cells and its impact on integrinb1 expression.
Materials and Methods: Wnt5A was overexpressed or knocked-down in SKOV-3 cells. Cells were stably transfected with pcDNA3.2/V5-DEST-Wnt5A construct or with pcDNA3.2/V5-DEST without Wnt5A (mock) by using Lipofectamine 3000. Wnt5A was knocked down using specific small interfering RNA (siRNA) against Wnt5A, and as negative controls non-target siRNAs (scramble) and transfection reagent (TR, Lipofectamine 2000) were only used. Following transfections, expression of Wnt5A was assessed by real-time qPCR and western blot analysis. Integrinb1 expression was analyzed using real-time qPCR. Spheroids formation ability was evaluated by using hanging drop technique. Migration of Wnt5A overexpressed cells was assessed using scratch assay. Reproductive viability of overexpressed Wnt5A cells was determined by colony formation assay.
Results: Wnt5A was increased by 2.4-fold accompanied with increased compaction of spheroids. siRNA Wnt5A transfected cells showed 70% decreased Wnt5A expression compared with scramble or TR which led to decreased spheroid growth and compaction. Migration and colony formation in Wnt5A overexpressed cells were increased compared to mock. In addition, Wnt5A overexpressed cells showed 8.7-fold increased integrinb1 expression compared to mock.
Conclusion: Our results suggest that Wnt5A may promote ovarian cancer cells spheroid formation and compaction which requires up-regulation of integrins.
***Keywords*:** Ovarian Cancer, Wnt5A, Spheroid, Integrinb1, Migration.

Ps-7: A Novel Electroactive Polyurethane Scaffold for Cardiac Tissue Engineering

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Objective: Our aim was to investigate the potential of electroactive polyurethane scaffold for cardiac tissue engineering.
Materials and Methods: A novel conductive polyurethane containing aniline pentamer (AP) was blended with polycaprolactone (PCL) and further fabricated into scaffolds using double porogen particulate leaching and compression molding method. Physico-chemical characterizations of the blend scaffold (PB) were evaluated by scanning electron microscope, porosity measurements, mechanical analysis and electrical conductivity measurements. For cell evaluations, cardiomyocytes were isolated from 1-2 days old newborn Wistar rats. Cell seeded scaffolds were assessed by immunofluorescent staining for comparative evaluation of cardiomyocyte specific proteins, including troponin T (TrpT) and connexin43 (Conx43) .Gene expression profiles of the cardiac markers including TrpT-2, Conx43 and Actinin alpha4 (Actn4) were analyzed by quantitative real-time PCR. All the results were compared with the non conductive PCL scaffold.
Results: Our results showed a consistent porous morphology all through both scaffolds with the interconnected pores throughout the whole structure ranging from several µm to 150 µm. PB scaffold had compression modulus and strength of 4.1 and 1.3 MPa, respectively. The conductivity of the scaffold was measured

as 10-5 ± 0.09 S/cm and preserved in culture medium for at least 100 h post fabrication. Scaffolds also supported neonatal cardiomyocytes adhesion and growth. Incorporation of AP led to the increased expression of the cardiac genes involved in muscle contraction and relaxation (troponin-T) and cytoskeleton alignment (actinin-4).
Conclusion: Our study highlighted the potential of embedding the electroactive moiety in the structure of the scaffold to direct electrical signals on cell activities for tissue engineering applications. Our data demonstrated that the inherently electrical conductive substrates are non-toxic and support cell proliferation and attachment.
***Keywords*:** Polyurethane, Cardiac Tissue Engineering, Conductive Polymers, Cardiomyocytes

Ps-8: Effects of Scaffold Architecture on Efficiency of Mechanical Stimulations of Mesenchymal Stem Cells Under Fluid Flow

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Objective: Mesenchymal stem cells (MSCs) are considered as multipotent stem cells which is mainly presented in bone marrow. Combining these cells with tissue engineering scaffolds is a promising method to regenerate tissues and organs. MSCs can be readily affected by cell culture conditions and biophysical stimuli. A dynamic culture -provided by a bioreactor, induces various mechanical stimuli on MSCs and in a long term- can influence proliferation, differentiation and cell fate determination. This investigation offers a finite element approach to define biophysical stimuli such as fluid shear stress and hydrodynamic pressure on cellular level and consequently the results can be used to predict cell fate as well.
Materials and Methods: In this study, using computer-aided design tools, two different scaffold architectures -based on implicit surfaces- were modeled. The single cell assuming the MSCs mechanical properties with idealized geometry was designed and attached in different positions on the scaffolds. Using tools of computational fluid dynamics and employing 1.2 million tetrahedral and prism elements, inlet fluid velocities of 50 and 100 μm/s were applied to simulate dynamic culture. Further, the solution satisfied mesh independency and accuracy.
Results: The results disclosed that a change in single cell position altered the magnitudes of shear stresses and hydrodynamic pressures. Furthermore, shift in scaffold architectures resulted in different fluid shear stress and hydrodynamic patterns on the single cell. Based on the results, the scaffold perpendicular channels with respect to fluid flow provided adequate conditions for cell culture. The models specified distribution of effective shear stresses and hydrodynamic pressure on both scaffolds and the single cell.
Conclusion: Current study showed that in particular fluid flow, there are specific surfaces in every scaffold transmitting optimal mechanical stimuli. Considering these crucial factors help researchers to modify the dynamic culture systems in order to set up more functional experiments *in vitro*.
***Keywords*:** MSCs, FSI, Mechanical Stimuli, Scaffold Architecture

Ps-9: A Rapid, Simple and Economical Method for The Isolation of Mesenchymal Stem Cells from Wharton's Jelly by Phosphate Buffer Saline

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Objective: The isolation of mesenchymal stem cells (MSCs) from the Wharton's jelly (WJ) without degradation of cellular surface receptors and protection cellular function, proliferation and viability is important for research and clinical applications. In this study we have established a simple, rapid protocol without enzymatic treatment in less time to isolate MSCs from WJ.
Materials and Methods: Human umbilical cords were collected after full-term deliveries and transported to the laboratory in sterile phosphate-buffered saline (PBS). Umbilical cords were cut into segments of 1–2 cm and removed cords vessels. The WJ sectioned were transferred into PBS and put on a shaker for 2 hours. The cord segments were discarded and the suspension was centrifuged and cultured in DMEM-LG supplemented with 10% fetal bovine serum for 5 days. The plastic adherent cells were investigated for surface markers CD90, CD105, CD73, CD44, HLA-DR, CD34/45, and CD133. Adipogenic, osteogenic, and chondrogenic differentiations were performed to investigate mesenchymal nature.
Results: After 5 days, purified populations of spindle-shape MSCs were appeared and the cells then expanded until they reached subconfluence . The expanded cells were positive for CD90, CD105, CD73, CD44 , and negative for CD34/45, CD133 and HLA-DR surface markers. WJ-MSCs showed multilineage cell differentiation potential into adipogenic, osteogenic and chondrogenic phenotypes.
Conclusion: This study indicates that this non-enzymatic protocol can result in efficient isolation of MSCs from WJ with less time. The expanded cells expressed characteristic markers and presented typical functional properties of MSCs such as differentiation capacities.
Keywords: Mesenchymal Stem Cells, Wharton's Jelly, Phosphate Buffer Saline

Ps-10: Effect of Digoxin on Mesenchymal Stem Cell Proliferation and Apoptosis

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Objective: Cardiac glycosides -such as digoxin or digitoxin- are the natural products that traditionally used to increase cardiac contractile force in patients with heart failure and cardiac arrhythmia. It has been shown that digoxin can directly inhibit the cell proliferation and lead to cell apoptosis. Present study was conducted to analyze the effect of digoxin in the cohorts of mesenchymal stem cells (MSCs) -based therapy.
Materials and Methods: MSCs were cultured and treated with different concentrations (0.1, 0.5, 1, 5, 7, 10, 15, 20, 30 and 40 μM) of digoxin for 6, 12, 24 and 48 hours. MTT assay was performed for cell viability and proliferation study. Also, to evaluate cell apoptosis a TUNEL assay was conducted.
Results: After 24 and 48 hours, cell viability was significantly decreased in 20, 30 and 40 μM concentrations ($P<0.05$), but significant decline in cell viability was observed only in 40 μM after 12 hours ($P<0.05$). After 6 hours, cell viability of experimental groups had no significant differences toward control group ($P>0.05$). In addition, cell apoptosis was increased along with rise in drug concentration and time. After 24 and 48 hours, cell apoptosis in 10 μM, 20 μM, 30 μM and 40 μM concentrations were higher than control group, significantly ($P<0.05$).
Conclusion: According to our obtained results we suggest that digoxin may lead to decline in cell survival ability and increase in cell apoptosis in a dose/time dependent pattern.
Keywords: Digoxin, Mesenchymal Stem Cell, Apoptosis, Cell Proliferation

Ps-11: Nanofiber-Expanded Human Umbilical Cord Blood-Derived CD34+ Cell Therapy Accelerates Murine Cutaneous Wound Closure by Attenuating Pro-Inflammatory Factors And Secreting IL-10

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Objective: Nanofiber-expanded human umbilical cord blood-derived CD34+ cell therapy is under consideration for treating peripheral and cardiac ischemia. However, the therapeutic efficacy of nanofiber-expanded human umbilical cord blood-derived (NEHUCB) CD34+ cell therapy for wound healing and its mechanisms are yet to be established.
Materials and Methods: Human umbilical cord blood stem cells were isolated from freshly collected samples, and were expanded using novel nanofiber mediated expansion technology. Expanded cells characteristics were determined by flowcytometry. Cuteneous wound were developed in immunocompromised NOD/SCID mice. Nanofiber-expanded CD34+ stem cells were injected to the mice using lateral tail vein and healing were measured. Cytoking and growth factor expression was measured by Real-time PCR methods from wound tissues. Histological analysis were performed to determine wound healing mechanisms. Finally mechanism confirmed using *in vitro* methods with human primary fibrobalst cells and stem cells.
Results: Using an excision wound model in NOD/SCID mice, we show herein that NEHUCB-CD34+ cells home to the wound site and significantly accelerate the wound-healing process compared to vehicle-treated control. Histological analysis reveals that accelerated wound closure is associated with the re-epithelialization and increased angiogenesis. Additionally, NEHUCB-CD34+ cell-therapy decreases expression of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and NOS2A in the wound bed, and concomitantly increased expression of IL-10 compared to vehicle-treated control. These findings were recapitulated *in vitro* using primary dermal fibroblasts and NEHUCB-CD34+ cells. Moreover, NEHUCB-CD34+ cells attenuate NF-κB activation and nuclear translocation in dermal fibroblasts through enhanced secretion of IL-10, which is known to bind to NF-κB and suppress transcriptional activity.
Conclusion: Collectively, these data provide novel mechanistic evidence of NEHUCB-CD34+ cell-mediated accelerated wound healing.
Keywords: NEHUCB-CD34+ Cells, Cutaneous Wound, NOD/SCID Mice, NF-κB, IL-10

Ps-12: Investigating The Behavior of A Dual Promoter System Containing Spermatogonial Specific Genes in The Presence of Extrinsic Factors

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Objective: Germ cells are responsible for the origin of new organisms and guarantee genetic continuity across the generations. Spermatogonial stem cells (SSCs) are a type of germ cells with unlimited proliferative poten-

tial. Their pluripotency and lower ethical concerns have made them the most suitable candidates for biotechnological, medicinal and transgenic studies as a substitute for embryonic stem cells.
Materials and Methods: In this study, we designed a dual promoter based lentiviral vector in order to track the simultaneous activity of Stra8 and c-kit promoters monitored by expression analysis of their downstream reporter genes (ZsGreen and DsRed2, respectively). The functional activity of promoters engineered in this construct was evaluated by transfecting GC-1 spg germ cell line when cells were under-treatment with RA, testis extract and CHIR99021 (a reprograming factor) using flow cytometry and real-time PCR approaches.
Results: We observed a different dynamic behavior of both promoters in the presence of extrinsic factors. The efficiency and accuracy of construct promoters in the presence of CHIR99021 were also analyzed and found to be comparable to the genomic endogenous promoters.
Conclusion: Our designed vector with the advantage of having all the genetic regulatory elements in a unique construct, could report the genetic responses to the exogenous stimuli. Further studies regarding multiple promoters expression would be required to engineer lentiviral vectors as functional tools for tracking the developmental events in germ cells.
Keywords: Reporter Construct, Dual Promoters, Lentiviral Vectors, Spermatogenesis

Ps-13: Purification and Transplantation of Human Embryonic Stem Cell Derived Midbrain Dopaminergic Progenitor Cells into The Rat Model of Parkinson's Disease

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Objective: Human embryonic stem cells (hESCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy in Parkinson's disease.
Materials and Methods: In this study we used a defined protocol to generate human DA progenitor cells from human embryonic stem cells that is based on dual SMAD inhibition and embryoid body (EB) formation.
Results: Our results showed that the human DA progenitor cells expressed the midbrain DA progenitor markers,FOXA2, CORIN, LMX1A and LMX1B and can be successfully differentiate into TH+ mature neurons. We surveyed the proteome of the human DA progenitor cells for novel surface proteins in previous study which resulted in expression of CNTN2, Flot2 and Calretinin. Flowcytometry analysis of these cells indicated that they express 20% CNTN2, 17% Flot2, 11% Calretinin and 22% Corin. To evaluate the functionality of these cells, we transplanted CNTN2+ cells in day 12 post neural induction into 6-OHDA-lesioned rats and also transplanted unsorted DA progenitor cells and human dermal fibroblasts (HDF) as control. The behavioral results of motor performance tests, such as apomorphine-induced rotation and cylinder test, showed that CNTN2+ cells can significantly improve motor behavior from week 2, compared to unsorted cells.
Conclusion: Our method is favorable in terms of efficiency and safety indicating promise approach for the development of cell-based therapies in Parkinson's disease.
Keywords: Embryonic Stem Cell, Dopaminergic neurons, Parkinson's Disease

Ps-14: The Role of bFGF, BMP4 and Noggin in Cardiomyocyte Differentiation of Human Adipose Tissue-Derived Stem Cells

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Objective: The aim of the present study was to evaluate the role of bFGF, BMP4 and noggin on cardiomyocyte differentiation from human adipose tissue-derived stem cells (ADSCs).
Materials and Methods: Human ADSCs were isolated from the adipose tissue samples of patients who underwent abdominoplasty. ADSCs were characterized by flow cytometry analysis. Third-passaged ADSCs were cultured to reach 100% confluency. Cardiac differentiation was induced by 4-day incubation of the ADSCs in DMEM plus 10% fetal bovine serum (FBS) or B27 (1X), 10 ng/ml bFGF and 10, 20 or 50 ng/ml BMP-4. After this initial stage, differentiation was continued in FBS-containing medium, and cardiac differentiation was examined after 21 days. The expression of cardiac transcription factors and cardiac-specific genes was evaluated by RT-PCR, quantitative real-time PCR, immunocytochemistry, flow cytometry and western blot analyses.
Results: Three-week differentiated ADSCs showed the expression of cardiac transcription factors, GATA4 and MEF2C, and cardiac-specific genes, α-MHC, β-MHC, MLC2V, MLC2A and ANP. The expression of MLC2V and ANP mRNAs were at their highest level when the

initial phase of differentiation was performed in FBS-containing DMEM and at the presence of 10 ng/ml bFGF and 20 ng/ml BMP-4. Immunocytochemical analyses showed the expression of α-actinin and cardiac troponin I proteins in the ADSC-derived cardiomyocytes. As revealed by flow cytometry analysis, a significant proportion of the differentiated cells were stained positively for cardiac troponin I, and western blot analysis confirmed the expression of Connexin43, α-actinin and Desmin proteins.
Conclusion: The results of present study indicate an important role for bFGF and BMP-4 in cardiomyocyte differentiation from ADSCs. Moreover, cardiac differentiation was more effective when the initial phase of differentiation was performed in FBS-containing medium.
Keywords: BMP-4, Human ADSCs, Differentiation, Cardiomyocyte

Ps-15: The Effect of Hypoxia during Differentiation of Human Embryonic Stem Cell Spheres to Hepatospheres in Spinner Flask

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Objective: Integrated scale up production of hepatocytes from hESC spheres (hESpheres) in spinner flask will facilitate the application of hepatocytes in drug screening assays and in bioartificial livers. But there are some problems in spinner flask including its large volume and low surface area which can lead to reduction in dissolved oxygen and therefore hypoxia will increase in this condition which can affect the efficient differentiation of hESpheres to hepatocytes spheres (hepatospheres).
In this study, we explored the role of hypoxia in comparison with normoxia during differentiation of hESpheres to hepatospheres in suspension dynamic culture.
Materials and Methods: hESpheres by adding different growth factors were differentiated to hepatospheres in spinner flask. The morphology, gene and protein expression of differentiated cells were compared under normoxia and hypoxia conditions.
Results: The cystic hepatospheres increased under hypoxia condition (30%) compared to normoxia (8%). The total sizes of hepatospheres under hypoxia condition were smaller than normoxia. Hepatospheres with ALB-positive cells under hypoxia was less than normoxia condition (47 and 75%, respectively). The expression of AFP and AAT as early markers in hepatospheres, under hypoxia was more than normoxia condition.
Conclusion: Results showed that hypoxia reduces the efficiency of hepatocytes production from hESpheres in spinner flask. To achieve efficient integrated large scale differentiation of hESpheres to hepatospheres, it is necessary to develop large scale differentiation in computer-controlled bioreactor instead of uncontrolled spinner flasks. Computer-controlled bioreactors may help to produce high quality hepatocytes with appropriate quantity for drug screening assays or bioartificial livers.
Keywords: Hypoxia, hESpheres, Stepwise Differentiation, Hepatospheres, Spinner Flask

Ps-16: Simple, Efficient and Cost-Benefit Protocol for Differentiation of Human Embryonic Stem Cell Spheres to Endodermal Cells

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Objective: Endoderm lineage is one of the important layers during human embryogenesis. Most cells in gut tube and its appendix -such as liver and pancreas- are derived from endodermal cells. Efficient and large scale production of endodermal cells as well as their derivation in-vitro are very important for drug screening assays, proteome analysis, artificial organ development in-vitro and animal model assessments. Therefore, development of a high efficiency and cost-benefit protocol for scale up production of these cells from human embryonic stem cells (hESCs) is very essential.
In this study for the first time, we developed a new simple, efficient and cost-benefit protocol for large scale production of endodermal cells from hESCs in suspension culture.
Materials and Methods: Pluripotent hESpheres were differentiated to mesoendodermal cells at the first step, then to endodermal cells. During activin treatment, the concentration of this component and competency of endodermal cells for differentiation to hepatocytes were evaluated. We also assessed the endodermal cells and hepatocytes production potential of this new protocol at large scale in spinner flask.
Results: In this study, it has been shown when hESpheres pretreated with high dose of WNT agonist, CHIR, followed by short term treatment -two days- in low concentration of activin; they were differentiated efficiently to endodermal cells in both static and dynamic suspension culture.
Conclusion: Pretreatment of hESpheres with high WNT agonist dose followed by low activin treatment was sufficient for differentiation of hESCs to endodermal cells. The endodermal cells produced by this new protocol are useful tools for scale up production of hepatocytes or B-cells for other studies.

Keywords: Human Embryonic Stem Cell Spheres, Differentiation, Endoderm

Ps-17: The Effect of bFGF and Fetal Calf Serum on Proliferation of Chicken Primordial Germ Cells and Stromal Cells

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Objective: Chicken gonadal tissue has two cell populations, incuding primordial germ cells (PGCs) and stromal cells (somatic cells). PGCs are suitable source for production of chicken pluripotent stem cell lines in transgenic birds, and subsequently vaccine and recombinant protein productions. In general, the effect of growth factors such bFGF, mLIF and serum for proliferative capacity *in vitro* are clear and important.
Materials and Methods: After incubation of fertilized chicken egg up to 6 days and isolation of primary gonadal tissues and culturing mixture of the cells -PGCs and stromal cells- these somatic cells proliferate in present of fetal calf serum (FCS) and bFGF, as two important factors for PGC culture. Somatic cells produce a multilayer feeder under the PGCs in primary culture and PGCs make a small cluster under these cells.
Results: In presence of growth factor and high volume of FCS (15%), only up to 4 or 5 days PGCs could be observed; while after that the stromal cells proliferate more than PGCs and cause PGCs disappearance.
Conclusion: Until now, we have seen many researches about derivation and maintenance of chicken PGCs, with hopes to understand the mechanisms that occur during germline development and produce therapeutic agents by transgenic birds. There are still many unknown questions in this area and this project will try to have efficient conditions for identification of suitable culture medium for long term culture of PGCs *in vitro* without serum and feeder cells.
Keywords: Chicken pluripotent Stem Cell Lines, Chicken gonadal Primordial Germ Cells, Proliferation, Growth Factors, Feeder Layer

Ps-18: Preconditioning of Mesenchymal Stem Cells with Hydrogen Peroxide and Serum Deprivation

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Objective: Because of plasticity and sensitivity of mesenchymal stem cells (MSCs), MSCs-based therapy faces with some limitations such as low proliferation rate of MSCs and cell death. In this regard, we evaluated the effect of preconditioning of mesenchymal stem cells with serum deprivation and H_2O_2 on cell proliferation, viability and apoptosis.
Materials and Methods: MSCs were cultured and preconditioned with serum deprivation and H_2O_2 for 6, 12, 24 and 48 hours in 6 groups (Control, I: 5μM H_2O_2, II: 10μM H_2O_2, III: 5%FBS, IV: 5μM H_2O_2+5%FBS and V: 10μM H_2O_2+5%FBS). After these time periods of treatment, the MSCs were exposed to lethal dose of H_2O_2 (300 μM) for 24 hours. Then, MTT assay and trypan blue staining were conducted to evaluate the cell proliferation and viability. TUNEL assay was also done to study the cell apoptosis.
Results: According to our data, cell proliferation in groups IV and V were increased significantly compared to the other groups after 6, 12 and 24 hours of treatment ($P<0.05$). After 48 hours, cell treatment with 5μM H_2O_2+ 5% FBS lead to significant increase in cell proliferation ($P<0.05$). The cell viability study showed significant increase in II, III, IV and V compared two other groups after 48 hours of treatment ($P<0.05$). Moreover, cell apoptosis of groups IV and V were significantly lower than control group after 12, 24 and 48 hours ($P<0.05$).
Conclusion: Our findings suggest that preconditioning with 5μM H_2O_2+5%FBS and 10μM H_2O_2+5%FBS can improve the cell proliferation and viability as well as lead to decline in cell apoptosis.
***Keywords*:** Mesenchymal Stem Cells, Hydrogen Peroxide, Serum Deprivation

Ps-19: The Oxytocin Is A More Potent Inducer of Cardiomyocytes Differentiation from Embryonic Carcinoma Cells P19 than 5-Azacytidine

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Objective: Capability of embryonic carcinoma cells P19 in differentiation to cardiomyocyte had examined through inducing effects of Oxytocin (OT) and 5-Azacytidin (5Az) individually and compared with each other *in vitro* condition.
Materials and Methods: Embryoid bodies (EBs) formed through hanging drops method. Then EBs were treated with (5Az) or (OT) as well as the EB medium

(Ctrl) until 12 days. The medium was renewed every 2 days. Morphology and beating number per minute were recorded every two days by invert-microscopy. Viability was carried out by trypan blue every three days. The expression of several cardiomyocyte-associated genes was assessed in 7 days EBs by RT-PCR.
Results: The beating area percentage of EBs in OT treatments group was more than 5Az group in all days of experiment. However, only in final stage significant increase in beating area of OT group was observed. There was no significant difference in viability and morphological changes between two groups. OT induction expressed three more specific proteins such as α–actinin, atrial natriuretic factor (ANF) and cardiac troponin T (cTnT) in cell culture than 5Az.
Conclusion: Statistical analysis in present study revealed response to OT inducer is more excessive than 5Az and EBs beating area percentage is significantly increased in OT treatment particularly final stage.
***Keywords*:** P19 Cells, Embryoid Bodies, Cardiomyocytes, 5-Azacytidin, Oxytocin

Ps-20: PPAR γ/PGC-1α-FNDC5 Pathway Involvement in Cardiac Differentiation Rate of mESCs

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Objective: Consistent with necessity of the heart to high energy, the cardiac myocyte differentiation needs a quite high energy which is produced by mitochondria. Peroxisome proliferator-activated receptor (PPAR) γ co-activator 1α (PGC-1α) is a powerful transcriptional regulator of energetic pathways. PGC-1α is attached to PPARγ and accelerates the interaction of this protein with multiple transcription factors. In 2012, Spiegelman and his colleagues introduced Fndc5 as a PGC-1α dependent myokine that was secreted as irisin from the muscle into blood, which caused an increase in energy expenditure. Previous studies showed that transcript levels of Fndc5 were high in heart and skeletal muscles of adult mouse. Our group found that Fndc5 expression is increased during the process of cardiac differentiation of mouse embryonic stem cells (mESCs) similar to PGC1α
Materials and Methods: In order to determine the correlation between PGC1α and Fndc5 in cardiac cell differentiation of mESCs, we utilized specific PPARγ agonist and antagonist during precursor cells (CPCs) formation of cardiac differentiation.
Results: We assessed that a reduction in PGC1α expression, via treatment with GW9662 during CPCs formation stage, down-regulated FNDC5 transcript levels as well as mitochondrial markers which negatively influenced on the whole process of cardiac differentiation efficiency. On the other hand, increase PGC1α expression during CPCs formation stage via rosiglitazone treatment increases Fndc5 expression level and mitochondrial markers transcription levels which enhanced cardiac differentiation efficiency.
Conclusion: We concluded that PPARγ agonist and antagonist induced up and down-regulation of PGC1α and subsequently modulated the process of CPCs formation through an alteration in Fndc5 and mitochondrial markers expression.
Keywords: Cardiac Differentiation, FNDC5, GW9662, Rosiglitazone, PGC-1α

Ps-21: Characterization and Comparing Amniotic Fluid Stem Cells before and after Vitrification

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Objective: Recent researches have indicated that amniotic fluid stem cells can provide unique source for clinical applications and regenerative medicine. The aim of this study was to compare amniotic fluid derived stem cells (AFSCs) before and after vitrification/warming in optimized culture condition for efficient long-term storage for therapeutic aims.
Materials and Methods: The amniotic fluid samples were obtained with informed consent of 6 pregnant women during an amniocentesis procedure in Teaching-Alzahra hospital of Tabriz, Iran. Samples were centrifuged at 1200 rpm for 10 min to obtain a pellet. The cells were seeded in 6 well plates without feeder layers and cultured in optimized culture condition separately for 4th passages, then isolated stem cells were vitrified/warmed and compared for gene expression and specific CD markers in both fresh and vitrified conditions using RT-PCR and flowcytometry, repectively.
Results: The present study demonstrated that both fresh and vitrified AFSCs had high growth rates with stem

cell-like cell morphology in optimized culture condition and they showed adherent properties in plastic surface. Fresh/vitrified AFSCs showed positive expression of Oct4 and Nanog genes and negative expression of CD31and CD45 markers which were equal in both fresh and vitrified cells. In contrast, expression of CD44 and CD90 in fresh cells were more than vitrified cells.
Conclusion: Our findings indicated that vitrification/warming did not have any negative effects on specific markers and gene expression of AFSCs. We will further analyze our stem cells for differentiation abilities. Our data showed promising result for efficient long-term storage of AFDS cells for therapeutic aims in the future.
***Keywords*:** Amniotic Fluid Stem Cells, Fresh and Frozen Cells, Flowcytometry

Ps-22: Migration of Bone Marrow-Derived Very Small Embryonic-Like Stem Cells Toward The Injured Spinal Cord

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Objective: Different ways have been tested for stem cells delivery in cell therapy experiments. One of the most convenient ways of stem cells administration is intravenous infusion. Very small embryonic-like stem cells (VSELS) are a rare population of stem cells in bone marrow that show some embryonic and adult stem cell characters. Ischemic was associated with significant increase on the number of circulating VSELS in PB. we transplanted very small embryonic like stem cells by intravenous injection in order to clarify whether these transplanted cells migrate into the injured spinal cord lesion.
Materials and Methods: Mouse BM aspirated from femur and then VSELS were isolated with FACS and cultured on C2C12 feeder layer cells. After characterization with immunocytochemistry, labeled VSELS injected via tail vein to compression spinal cord injury model rats. 4 weeks after transplantation, tissue was assessed with fluorescence microscopy and homming was evaluated by real-time PCR analysis and histological study for identification of injected population in site of injury
Results: Findings show that Very small embryonic-like stem cells can be isolated from BM by FACS and expanded *in vitro*, revealed a large number of DiI-labelled cells in the lesion. Real-time PCR analysis confirmed that the expression of Oct4 mRNA was significantly increased in the injury site. SSEA1 antibody positive also demonstrated that VSELS had migrated into the lesion.
Conclusion: Very small embryonic-like stem cells identified in mice and the mobilization of VSELS into PB occurs in rat with spinal cord injury.
Keywords: VSELSC, Homming, Compression SCI

Ps-23: The Influence of Cerebrospinal Fluid Accompanied by Retinoic Acid on Differentiation of Bone Marrow Mesenchymal Stem Cells into Neuron-Like Cells *In Vitro*

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Objective: Cerebrospinal fluid (CSF) has a broad range of molecules, growth and neurotrophic factors which are essential for neurogenesis in embryos and adults. Bone marrow mesenchymal stem cells (BMSCs) are multipotent stem cells that can differentiate into the cells with neural-like phenotype under the induction of appropriate growth factors. According to the significant role of retinoic acid (RA) in neurogenesis, the aim of this study was to induce differentiation of BMSCs into neuron-like cells using CSF, RA and combinative form of CSF and RA.
Materials and Methods: Bone marrow mesenchymal stem cells were isolated and characterized. The CSF was prepared from cisterna magna of 19 days Wistar rat embryos. Then BMSCs were induced by 5% CSF (CSF group), 10-6 μM retinoic acid (RA group) and CSF plus RA (CSR group) for 12 days. Morphology of differentiated cells was examined by inverted microscope and axonal outgrowth was measured using the image J software. In addition, the expression of neural-specific markers (Nestin and MAP-2) was examined by Immunocytochemistry.
Results: The specific - neuronal morphology was observed in differentiated cells. The maximum axon length was seen in CSR group on 12th day of induction. The results of immunocytochemistry showed the expression of Nestin, a neuroprogenitor marker, in all treated groups. However, MAP-2, known as a mature neural marker, was expressed only in CSR group.
Conclusion: According to the findings of this study, the synergistic effect of CSF and RA enhances the differentiation of BMSCs into neuronal and glial phenotypes *in vitro*.
***Keywords*:** BMSCs, CSF, RA, Neuron-Like Cells, Differentiation

Ps-24: The Effect of Cyclic Uniaxial Stretch on Tenogenic Differentiation of Rabbit Adipose-Derived Mesenchymal Stem Cells

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Objective: Tissue engineering using stem cells is a promising method for tendon regeneration. Cyclic stretch is one of the important factors affecting differentiation of mesenchymal stem cells (MSCs) into tenocytes.Utilization of such mechanical signal may improve tenogenic differentiation of stem cells *in vitro* and can be used in tendon tissue engineering applications.
Materials and Methods: The aim of this work was to study the effect of cyclic strain on tenogenic differentiation of rabbit adipose-derived mesenchymal stem cells (rADSCs). After isolation of rADSCs from abdominal fat tissue, characterization by flow cytometry and assessment of multilineage differentiation potential, pellets of passage 3 cells were cultivated on a PolyDiMethylSiloxane (PDMS) substrate. A cyclic uniaxial stretch device, which had been previously developed in National Cell Bank of Iran, Pasteur Institute of Iran, was used for mechanical stimulation.The substrate was subjected to cyclic stretch (10% strain, 1 Hz frequency) for 24 h. Real-time PCR method was used to examine the expression of collagen type I (col I) and decorin as two important tenogenic markers.
Results: Regarding flow cytometry results, expression levels of cell surface proteins were highly characteristic of MSCs. Multipotency of isolated rADSCs was confirmed by their multilineage differentiation. According to real-time PCR results, application of cyclic uniaxial stretch increased the expression of Col I (by 6 folds) and Decorin (by 1.5 folds) compared to the control group.
Conclusion: The higher expression of Col I and Decorin, in response to mechanical stretch, indicates the positive effect of such mechanical signal on tenogenesis and that uniaxial stretch can be used as an effective tool in rADSC stenogenic differentiation and tendon tissue engineering.
Keywords: Mesenchymal Stem Cells, Uniaxial Stretch, Tenogenic Differentiation

Ps-25: Investigation of von Willebrand Factor (vWF) Gene Expression in Endothelial Cells Differentiated from Adipose-Drived Mesenchymal Stem Cells and its Stability

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Objective: Vascular endothelial cells are subjected to shear and tensile stresses induced by blood flow and pressure, respectively. Cells assigned to cover the tissue engineered vessels have been already isolated from several human sources such as blood vessels, bone marrow, and recently adipose tissue. There have been studies concerning the differentiation of adult stem cells into ECs, mostly using mesenchymal stem cells (MSCs). When subjected to different mechanical stimulations, MSC differentiation varies. For example, Fluid shear stress can induce their differentiation towards endothelial cells and the process is believed to play an important role in vasculogenesis and cardiac repair.
Materials and Methods: Human adipose-drived mesenchymal stem cells (AdMSCs), were characterized in passage 3 using flow cytometry. In order to mimic the *in vivo* conditions, AdMSCs were cultured in tubular silicone scaffolds, and then exposed to endothelial growth factor (VEGE) for 7 days or shear stress of 4/49 dyn/cm2 for 24 hours, using a perfusion bioreactor. In order to examine the differentiation, the expression level of vWF, as an endothelial specific gene, was studied using real-time PCR, and for studying the stability of differentiation, the mRNA levels of this gene were quantified, upon completion of each test, as well as on the 5th and 10th days after the test.
Results: According to real-time PCR results, immediately upon the completion of mechanical and chemical tests as well as on the 5th and 10th days after the experiments, the mRNA level of vWF was higher in the mechanical group (approximately 5.28, 1.18 and 2.97 respectively), compared to the chemical ones (approximately 1.1, 1.69 and 1.28 respectively).
Conclusion: The findings of this study indicate that shear stress alone can be an endothelial differentiation factor by itself. It was demonstrated that in 10 days after exposure of mesenchymal stem cells to shear stress and chemical signals, stability in the expression of vWF gene could be observed. The results of this study can be used as a tool to achieve a practical and stable endothelial differentiation of ADSCs, for application in cardiovascular treatments.
Keywords: Mesenchymal Stem Cells, Endothelial Cells, Cell Differentiation, Shear Stress, VEGF

Ps-26: The First Report of Avian Germline Cyst Formation in The Ostrich Embryo

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Objective: Primordial germ cells (PGCs) in many species initiate gametogenesis by formation of interconnected cells known as germline cysts. Recent studies have confirmed this conserved phase of PGCs development in males of Xenopus, mouse and Drosophila, but there is no report of cystogenesis in avian PGCs. Considering the only reported exception of germline cyst formation is C. elegans, this study was carried out to investigate the presence of germline cyst formation in the ostrich embryo as an avian model.
Materials and Methods: A total number of 50 ostrich eggs were incubated at 36-37°C temperature and 25 ± 2% relative humidity with tilting to 90° at 4 h intervals. Seven faulty eggs were discarded at the second week but the others were incubated for 20, 26, 30, 36 and 42 days. Left testicles of obtained embryos were dissected, photographed and fixed in formaldehyde or glutaraldehyde fixators for preparation of paraffin or resin sections. For light microscopy the sections were stained by H&E, PAS, Masson's trichrome, Alcian blue and toluidine blue.
Results: In the paraffin sections of 26, 30, 36 and 42 days old embryos, a few aggregations of PGCs were observed. In the resin sections some aggregations of PGCs were thought to be connected to each other. In the resin sections the joint cytoplasm of these cells and interconnected cell boundaries were confirmed.
Conclusion: The phase of germline cyst formation is conserved in the ostrich similar to the mouse and Xenopus. Although ostrich PGCs localize to the genital ridge before the embryonic day 20, they seem to form multinucleated cysts some while after this day. Observation of germline cysts in the embryonic testis is not suitable using the paraffin sections, but in the accurately prepared resin sections they are readily recognizable.
Keywords: Germline Cyst, Germ Cell, Avian Embryo, Ostrich

Ps-27: Estrogen Receptor Beta: A Novel Transcription Factor for Aromatase Coding Gene (CYP19A1) Regulation in Cumulus Cells

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Objective: The pivotal functions of aromatase and its product intrafollicular estrogen in regulation of follicular growth and development are well known. Estrogen mainly mediates its effects in ovarian cells through estrogen receptor β (ERβ). ERβ can act in both up- and downregulation of target genes via recognizing binding sites on genome. Despite several reports focused on aromatase, the regulation of aromatase coding gene (*CYP19A1*) expression has not been extensively studied in human cumulus cells (CCs). *CYP19A1* is expressed in ovarian cells via an ovarian-specific promoter II (PII). Furthermore, given the ability of ERβ to binds to ERE half sites (estrogen responsive elements half sites) that exist in PI.3 and PI.4, we challenged the hypothesis that the ERβ may be a regulator of aromatase expression by investigate incorporation of ERβ on promoter regions of *CYP19A1* gene.
Materials and Methods: Cumulus oocyte complexes were obtained from 20 infertile patients with tubal factors or/and egg donor who underwent ovarian stimulation with GnRH agonist or antagonist for intracytoplasmic sperm injection. Only the CCs from MII oocytes were selected for this study. The association of ERβ with three promoter regions (PII, PI.3 and PI.4) of *CYP19A1* gene was examined using Chromatin Immunoprecipitation (ChIP) assay.
Results: Our data revealed that ERβ are incorporated into the three analyzed regulatory regions; however, in women with GnRH agonist protocol, its incorporation on promoter PII is significantly higher than other promoters. In contrast, in women with GnRH antagonist protocol, incorporation of ERβ on promoter PI.3 and PI.4 is significantly higher than promoter PII.
Conclusion: Our results have shown that human cumulus cell aromatase is controlled, in part, by locally produced estrogen hormone through its receptor (i.e., ERβ). This steroid hormone receptor can be added to the other regulatory transcription factors which direct the expression of the *CYP19A1* gene in human cumulus cells.
Keywords: Estrogen Receptor Beta, *CYP19A1*, Cumulus Cell

Ps-28: The Effects of Different Times Mesenchymal Stem Cells Injection on Brain Stroke in Rats

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Objective: Brain stroke is one of serious problems which leads to disability and morbidity. There is no effective treatment recovering neurological functions. Recently, cell therapy with mesenchymal stem cells transplantation opens a hopeful window to approach to brain stroke because of their neuro-protection and anti-apoptotic effects. In this study, we design an experiment to define the optimum time for injecting mesenchymal stem cells (MSCs) and get the best neurological outcome.
Materials and Methods: The MSCs were isolated from Sprague Dawley rats' tibias and femurs and they were expanded till passage 4. Some Mesenchymal stem cell markers were assessed for isolated cells including CD44, CD90, CD29 and CD34. The middle cerebral artery occlusion (MCAO) was performed to induce brain stroke. The MSCs were injected in different time containing 1 hour, 12 hours, 1 day, 3 days, 5 days and 7 days after MCAO. The neurological function and brain damage were evaluated for 28 days after MSCs transplantation.
Results: The isolated MSCs were positive for CD44, CD90, and CD29 and negative for CD34. The examination for neurological function shows that injection of MSCs in 12 hours after MCAO had the best effect through other groups and it has also the least brain volume damage.
Conclusion: The injection of MSCs in 12 hours after MCAO is an effective way to reduce the brain stroke consequences, in addition it makes brain damage less than the other time MSCs injection.
***Keywords*:** Mesenchymal Stem Cells, Brain Stroke, Different Times

Ps-29: A Novel Cell Therapy Method for Recovering after Brain Stroke in Rats

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Objective: Nowadays, brain stroke leads to a significant part of the adult mortality and morbidity and also it could result in some neurological deficits in the patients' lives. Cell therapy has opened a new approach to treat the brain ischemia and reduce its terrible effects on the patients' lives. There are several articles which show that the cell therapy could be beneficial for treating brain stroke. In this study, we have planned to present a new cell therapy method for brain stroke by administration of Mesenchymal stem cells and differentiated neural stem cells without astrocytes.
Materials and Methods: The mesenchymal stem cells (MSCs) were isolated from tibia and femur of a 250-300 g rat and they were cultured in DMEM/F12, 10% fetal bovine serum, 1% Pen/Strep. Neural stem cells were isolated from 14 days rat embryo ganglion eminence and were cultured in NSA media containing Neurobasal, 2 % B27, bFGF 10ng/ml and EGF 20 ng/ml after 5 days they formed some neurospheres. The isolated neural stem cells were differentiated to neural lineages by adding 5% fetal bovine serum to their culture media. After 48 hours the astrocytes were depleted by using MACS kit.
Results: The group that received MSCs systemically and differentiated neural stem cells without astrocytes had the best neurological outcomes and the least infarct volume and apoptosis. It could be understood that this cell therapy method might cause almost full recovery after brain stoke
Conclusion: Using combination cell therapy with MSCs and differentiated neural stem cells with removed astrocyte could provide a novel method for curing brain stroke
Keywords: Neural Stem Cells, Mesenchymal Stem Cells, Brain Stroke, Astrocyte

Ps-30: Combination Cell Therapy with Mesenchymal Stem Cells and Neural Stem Cells for Brain Stroke in Rats

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Objective: Brain stroke is the second most important events that lead to disability and morbidity these days. Although, stroke is important, there is no treatment for curing this problem. Nowadays, cell therapy has opened a new window for treating central nervous system disease. In this study, we have designed an experiment that the combination cell therapy mesenchymal and neural stem cells was used for brain stroke.
Materials and Methods: The mesenchymal stem cells (MSCs) were isolated from adult rat bone marrow and the neural stem cells were isolated from ganglion eminence of rat embryo 14 days. The MSCs were injected 1 day after middle cerebral artery occlusion (MCAO) and the neural stem cells transplanted 7 days after MCAO. After 28 days, the neurological outcomes and brain le-

sion volumes were evaluated. Also, the activity of Caspase 3 was assessed in different groups.
Results: The group which received combination cell therapy had better neurological examination and less brain lesion. Also the combination cell therapy group had the least Caspase 3 activity among the groups.
Conclusion: MSCs therapy is more effective than Mesenchymal stem cell therapy and neural stem cell therapy separately for treating the brain stroke in rats.
Keywords: Mesenchymal Stem Cells, Neural Stem Cells, Combination Cell Therapy, Brain Stroke

Ps-31: Behavior of Human Induced Pluripotent Stem Cells on Electrospun PCL Nanofibers and PCL Film

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Objective: Extracellular matrix (ECM) components play a critical role in regulating cell behaviors. Interactions between ECM components and cells are important in various biological processes, including cell attachment, survival, morphogenesis, spreading, proliferation, and gene expression. In this study the *in vitro* responses of human induced pluripotent stem cells (hiPSCs) on polycaprolactone (PCL) electrospun nanofibrous scaffold were reported in comparison with those of the cells on corresponding solution-cast film scaffold.
Materials and Methods: The properties of the scaffolds were investigated by fourier transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) and The resulting cells were analyzed for adhesion genes and protein expression by qRT-PCR and immunocytochemistry.
Results: Our results demonstrated that the nanofibrous scaffold showed better support for the attachment and proliferation of hiPSCs than their corresponding film scaffold. Indeed, toxicity test indicated that the electrospun nanofibrous scaffold was more biocompatible than PCL solution-cast film scaffold and nanofibrous scaffold could significantly improve the cell viability of hiPSCs.
Conclusion: Consequently, we emphasize that hiPSCs can sense the physical properties and chemical composition of the materials and regulate their behaviors accordingly and Nanofiberous PCL scaffold can be used as a suitable broad spectrum scaffold for hiPSCs.
Keywords: Adhesion, Proliferation, hiPSCs, Electrospinning, Scaffold

Ps-32: miRNAs Expression Profile during Osteogenic Differentiation of Human Adipose-Derived Stem Cells Cultured on Aligned Biodegradable Electrospun Nanofibrous Scaffold

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Objective: Bone tissue engineering is an interesting approach for the treatment of Bone fractures, osteoporosis, malformation, and clinically important bone diseases. Electrospun poly-L-lactide (PLLA) aligned nanofibers improve osteogenic differentiation of mesenchymal stem cells because of their niche-mimicking features exploited to regulate stem cell attachment, proliferation, differentiation and function *in vitro* and *in vivo*. Osteoblast differentiation is tightly regulated by post transcriptional regulators such as microRNAs; thus controlling miRNA activity in hMSCs can be an effective tool for enhancing the induction of osteogenesis for tissue engineering purposes.
Materials and Methods: In this study, aligned PLLA nanofibrus scaffolds were fabricated using electrospinning technique. Human adipose tissue derived stem cells (hAD-MSCs) were seeded on aligned electrospun fibrous with an initial cell density of 2×10^5 cells per cm^2 and incubated in the osteogenic medium for 21 days. Then we investigated the miRNA expression profile during osteogenic differentiation of hADSCs, and assessed the role of nanofibrous topographies on the osteogenic differentiation potential of hADSCs in comparison with TCPS. Putative targets of the miRNAs were predicted using online software miRanda, TargetScan, miRBase which identified its regulatory network, molecular function, biological processes and its target genes involved in negative regulation of osteoblast differentiation. miRNA expression profiles during osteogenic differentiation of MSCs were verified using quantitative real-time polymerase chain reaction (qRT-PCR).
Furthermore,TGF-beta signaling pathway activity has been analyzed as an important regulatory pathway of osteogenesis.
After 7, 14, 21 days osteogenic commitment of osteoblasts was evaluated by measuring ALPase activity, extracellular calcium deposition ,specific staining and evaluation of some main osteogenic marker genes.
Results: These findings demonstrate that hADSCs, cultured on electrospun PLLA aligned fibers, had higher ALP activity and calcium deposition in comparison with Tcps. Thus aligned nanofibrus provides a good en-

vironment for cell growth and osteogenic differentiation and have great potential in the bone tissue engineering. Furthermore, our study reveals more details about osteogenic differentiation of hADSCs and suggests controlling miRNA activity in hMSCs, as a novel biomarker, through effect of nanofibrus scaffolds on miRNAs profiling and pathways involved in osteogenesis. Taking together, it can be an effective tool for enhancing the induction of osteogenesis for tissue engineering purposes.
Conclusion: These results suggest that PLLA aligned nanofibers could influence on the expression of miRNAs, as a novel approach for controlling osteoblast differentiation.
***Keywords*:** microRNAs Profile, Electrospinning, Topography, Nanofiber, Osteogenic Differentiation

Ps-33: Characterization of Neural Stem Cells Isolated from Adult Rhesus Macaque Monkey's Subventricular Zone

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Objective: Neural stem cells of adult mammalian brain have the potential ability to be used as a source of tissue of autologous cell replacement strategies for the cell treatment of neurodegenerative diseases. Neural stem cells (NSCs) are self-renewable in culture and can generate mature neural progeny which display the characteristics of functional neurons and glia. This cell population could therefore be suitable source for cell therapy in the treatment of neurodegenerative disease. Our goal in this study was to characterize the neural stem cells isolated from subventricular zone.
Materials and Methods: The subventricular zone (SVZ) was microdissected from Rhesus Macaque monkey (male) and dissociated with trypsin, the single cell was cultured into NSC medium. Flow cytometry analysis, Immunofleoroscent staining and qRT-PCR were performed in the fourth passage. Karyotype of isolated neural stem cells from Monkey's SVZ was also checked. Furthermore, the ability of colony formation from single cell was evaluated.
Results: Immunofleoroscent staining and flow cytometry analysis of NSC-SVZ showed that these cells were $NESTIN^+$, $GFAP^+$, $SOX1^+$, $SOX2^+$ and $PAX6^+$ in the expansion of Neural stem cell medium. NESTIN, GFAP, SOX1, SOX2 and PAX6 showed high level of gene expression at the forth passage. Karyotype analysis showed a normal male chromosome complement (42, XY).
Conclusion: Our study demonstrated that the cells have characteristics of neural stem cells. It seems that these cells were suitable option for cell replacement of neurodegenerative diseases.
***Keywords*:** Neural Stem Cells, Subventricular Zone, Self-Renewal, Rhesus Macaque Monkey, Neurodegenerative Diseases

Ps-34: Evaluation of SOX9 Expression in 3D-Cultured Stem Cells Using A Specialized Cartilage Tissue Engineering Bioreactor

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Objective: Articular cartilage is an avascular tissue with limited intrinsic healing capacity. Among all possible cell sources for cartilage repair, mesenchymal stem cells (MSCs) have attracted a great deal of interest for such application. Matrix synthesis enhancement for cell-based cartilage regeneration aims at providing anchorage support to cells and localizing them in the injury site after implantation in order to prevent delamination and failure of the graft. It is becoming increasingly clear that differentiation of MSCs is controlled by mechanical signals. Mechanical forces which are generated within the cell in response to its surrounding extracellular matrix play major roles in determining cell fate. The aim of this study is to assess the effects of cyclic hydrostatic pressure on chondrogenesis of encapsulated MSCs.
Materials and Methods: Rabbit adipose-derived (AD)-MSCs and chondrocytes were obtained from National Cell Bank of Iran (Pasteur Institute of Iran) and at passage 4, they were encapsulated in alginate hydrogels and then incubated at 37°C for 0, 7 and 14 days. Thereafter, half of the samples were exposed to 5 MPa of cyclic hydrostatic pressure at 0.5 Hz for 7 days and the remaining samples were incubated at 37oC and used as the control group. Cellular toxicity, adhesion and proliferation of encapsulated cells were evaluated. The expression of SOX9 in encapsulated cells after hydrostatic pressure application was quantified using real-time PCR.
Results: According to cell adhesion and proliferation results, alginate capsules which were used for 3D cell culture resulted in higher cell densities and provided better microenvironments for cells, compared to 2D culture plates. Based on the MTT assay results, all cell viabilities in alginate capsules after 3, 7 and 14 days were greater than 90%.
The expression of Sox9, as an important chondrogenic marker, in MSCs was increased by 3.5 folds in response to the application of hydrostatic pressure, compared to

the control group. The expression of this gene was increased over time, so that it was 2 times higher on day 21 compared to that on day 7.
Conclusion: According to proliferation and MTT assays, alginate provides a good 3D environment for cells. The application of hydrostatic pressure on alginate-encapsulated MSCs increases the expression of Sox9, as a chondrogenic gene, and can be used in cartilage tissue engineering applications. Such alginate structure with differentiated MSCs can be injected to cartilage defects.
***Keywords*:** Hydrostatic Pressure, Hydrogel, Mesenchymal Stem Cells, Sox9, Chondrogenesis

Ps-35: Autologous Transplantation of Mesenchymal Stem Cells/Scaffold for Bone Regeneration of Osteomyelitis in A Rabbit Model

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Objective: Infection of bone (osteomyelitis) remains one of the most challenging problems in the field of orthopedic surgery. Currently, treatment consists of certain medications, surgical procedures such as debridement, and use of grafts. Such therapies have been reported to have limited abilities to treat osteomyelitis. Furthermore, they would be associated with a range of additional problems. On the other hand, mesenchymal stem cells (MSCs) have emerged as suitable cellular material for regeneration of large defects in bone tissue. To investigate therapeutic effects of MSC on osteomyelitis, one approach would be to experimentally create the osteomyelitis in animal model and transplant MSCs into the defect.
Materials and Methods: In current study, the osteomyelitis was experimentally induced in the legs of rabbit using injection of S. aureus. Then, the rabbits were divided into 4 experimental groups according to the treatment that they received: 1- antibiotic, 2- antibiotic+ scaffold+ fibrin glue, 3- antibiotic+ scaffold+ MSCs+ fibrin glue and 4- MSCs+ scaffold+ fibrin glue. Finally, the groups were compared using radiological images, as well as pathological and histological sections.
Results: According to our findings, there was a variation between different groups in terms of the disorder treatment. The third group has shown greater improvement than the other groups.
Conclusion: In general it could be concluded that the application of MSCs with antibiotics would be effective in treatment of osteomyelitis.

Keywords: Osteomyelitis, Bone Tissue Regeneration, Mesenchymal Stem Cells

Ps-36: Effect of 1α, 25-Dihydroxy Vitamin D3 on Osteogenic Differentiation of Stem Cells from Dental Pulp of Exfoliated Deciduous Teeth

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Objective: Stem cells from human exfoliated deciduous teeth (SHEDs) are a population of highly proliferative cells capable of differentiating into osteogenic, odontogenic, adipocytes and neural cells. Vitamin D3 metabolites such as 1α,25-dihydroxy vitamin D3 are key factors in the regulation of bone metabolism. The aim of this study was to investigate the effect of 1α,25-dihydroxy vitamin D3 on osteogenic differentiation (alkaline phosphatase activity and Alizarin red staining) of stem cells of exfoliated deciduous teeth.
Materials and Methods: Dental pulp was removed from freshly extracted primary teeth and immersed in a digestive solution. Then dental pulp cells were immersed in α-MEM culture medium, added with 10% FBS (fetal bovine serum). After the third passage, cells were isolated from the culture plate and were used for osteogenic differentiation. As a control group, cells were cultured in osteogenic cell culture medium and as a case group cells were cultured in osteogenic culture medium supplemented with 100 nM 1α,25(OH)2D3. Alkaline phosphatase (ALP) activity and alizarin red staining were analyzed to evaluate the osteogenic differentiation at day 21. The results were analyzed using t-test.
Results: Significant increase was in ALP activity of SHEDs after treatment with 1α,25(OH)2D3 in comparison to cells in control group (P=0.002). Alizarin red staining demonstrated that cells exposed to 1α,25(OH)2D3 induced higher mineralized nodules (P<0.001).
Conclusion: Osteoblast differentiation in SHEDs was stimulated by 1α,25(OH)2D3. Based on these findings, we conclude that 1α,25 (OH) 2D3 can be used as osteogenic inducer in tissue engineering.
Keywords: Stem Cells, Dental Pulp, Deciduous Tooth, 1α, 25-dihydroxyvitamin D3

Ps-37: Transplantation of Human Induced Neural Progenitor Cells to The Contusion Model of Rat Spinal Cord Injury

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Objective: Traumatic injury to the spinal cord causes cell death, demyelination, axonal degeneration, and cavitation resulting in functional motor and sensory loss. Stem cell therapy is a promising approach for spinal cord injury (SCI). Various cell types have been transplanted into the injured rat spinal cord and most of them resulted in functional recovery. Although neural progenitor cells (NPCs) had the best results because of the similarity of spinal cord to their favorable niche, the major problem is that they are not autologous.
Materials and Methods: In this study, we transplanted human induced neural progenitor cells (hiNPCs) which were trans-differentiated from adult human fibroblasts by the force defined transcription factors into the injured spinal cord of adult Wistar rats at 6 days after injury. Behavioral analyses were performed from the time of the initial injury until 7 weeks after SCI.
Results: Our results show the presence of a substantial number of surviving hiNPCs in the injured spinal cord up to 6 weeks after transplantation. Grafted hiNPCs survived and differentiated into the three major neural lineages (neurons, astrocytes, and oligodendrocytes). These cells also migrate to the rostral and caudal parts of the rat spinal cord. Furthermore, we observed that injured rats receiving hiNPC transplants had improved functional recovery as assessed by the Basso, Beattie, and Bresnahan Locomotor Rating Scale and grid-walk analysis.
Conclusion: Our data provide strong evidence in support of the feasibility of iNPCs for cell-based therapy in patient with SCI.
Keywords: Spinal Cord Injury, Trans-Differentiation, Induced Neural Progenitor Cell, Transplantation, Rat

Ps-38: The Necessity of Religious Jurisprudence and Juridical studies in Treatment of Stem Cells

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Objective: The stem cells are non-differentiated cells which can potentially produce every cell and tissue of human body. Therefore, the stem cells have special state in tissue engineering and renewal medicine. Using this technology in different societies concerns different juridical laws and regulations. According to noticeable success of our country in this field, it seems that it is necessary to prepare and compile special regulations and laws for this case ever-increasingly.
In this paper, in addition to rendering science generalities we will discuss Jurisprudence and Juridical of this regard.
Materials and Methods: Our method is so that we will evaluating different technical dimensions of problem. at first and second steps, we will investigate different jurisprudence and juridical reasons of the case and thirdly we will select some justifications for critique and evaluation.
Results: Goals, importance and the necessity of study:
1. Clearing present Jurisprudence and Juridical state of Iran's Stem Cells (present state)
2. Comparing the Islamic jurisprudence in the Imamia sect with the newest methods of stem cells' treatment and research.
3. Resolution of jurisprudence challenges concern with stem cells recovery and other resources of its producing.
4. Recommend of some juridical laws and regulations in order to:
1. Recovery of embryonic stem cells for research and treatment
2. Using of adult stem cells for research and treatment
3. Resolution of adverse events and dangers of other application of stem cells
4. Supervising laws for research centers and institutions.
Conclusion: We hope that this paper prepares the substrate of compiling different enactments and laws in this area and trial dangers/disadvantages versus advantages in order to enter in health and treatment field via this case.
***Keywords*:** Stem Cells, Shiites' Jurisprudence, Juridical Bases

Ps-39: Hypoxic Preconditioning Protects Bone Marrow Mesenchymal Stem Cells against Induced Apoptosis and Enhances Cell Proliferation and Viability

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Objective: Poor cell survival ability and limited functional benefits have restricted the efficacy of bone marrow mesenchymal stem cells (BMMSCs) in cell therapy. Recently, it has been reported that hypoxia preconditioning of BMMSCs *in vitro* before transplantation can enhance the survival and therapeutic properties of these cells. Present study investigated the effect hypoxic preconditioning of mesenchymal stem cells with hydrogen peroxide with (H_2O_2) and cobalt chloride ($CoCl_2$) on cells proliferation, viability and apoptosis *in vitro*.
Materials and Methods: Bone marrow mesenchymal stem cells preconditioned with H_2O_2, $CoCl_2$ as hypoxia mimetic agent and both H_2O_2, $CoCl_2$ for 6, 12, 24 and

48 hours. Then, the MSCs were exposed to lethal dose of H_2O_2 (300 μM) for 24 hours followed by 24 hours recovery. MTT assay and trypan blue staining were conducted to evaluate the cell proliferation and viability. Also, TUNEL assay was done to study the cell apoptosis.
Results: According to our data, cell proliferation in group preconditioned groups increased significantly compared to the other groups after 6, 12, 24 and 48 hours ($P<0.05$). Cell survival significantly increased in preconditioned groups with H_2O_2 and hypoxia after 6, 12, 24 and 48 hours ($P<0.05$). Moreover, cell apoptosis was decreased in preconditioned groups significantly than control group after 6, 12, 24 and 48 hours ($P<0.05$).
Conclusion: This study clearly demonstrated that hypoxic preconditioning with H_2O_2 and $CoCl_2$ can improve the cell proliferation and viability as well as lead to decline in cell apoptosis.
Keywords: Mesenchymal Stem Cells, Hypoxic Preconditioning, Hydrogen Peroxide, Cobalt Chloride

Ps-40: Synergistic Effects of Temozolomide and Thymoquinone on U87MG Cell Line

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Objective: The alkylating agent temozolomide is the major chemotherapeutic drug used clinically in the treatment of glioblastoma multiforme. Glioblastoma is still associated with a poor prognosis, because after first treatment it recurs, and then, displays resistance to further temozolomide treatment. Therefore, resistance is a major obstacle to glioblastoma therapy. Studies show that the combination therapy with natural substances can enhance the anticancer activity of temozolomide. Thymoquinone is a bioactive ingredient from Nigella sativa and has been investigated for its anticancer activities in a variety of cancer cell lines. This study investigated the mechanism behind temozolomide-induced cell death and the possibility that thymoquinone might increase temozolomide efficacy.
Materials and Methods: Dose response study was performed to determine the suitable doses of each drug for using in these experiments. U87MG cells were treated with 10, 20, 50 and 100 μM temozolomide and 10, 20, 50, 100, 150 and 200 μM thymoquinone alone, and also in combination of 20 μM temozolomide with 10, 20, 50, 100, 150 and 200 μM thymoquinone for MTT assay. For other tests, concentration of 20 μM temozolomide and/or 50 μM thymoquinone were chosen. For quantification of apoptosis, necrosis and autophagy, the cells were acridine orange ethidium bromide stained and detection of acidic vesicular organelles was performed. Data were analyzed by one-way ANOVA and $P<0.05$ was considered significant.
Results: Temozolomide and thymoquinone, alone and in combination, decreased significantly cell viability in U87MG cells, in a concentration and time dependent manner. Temozolomide induced both apoptotic cell death and cytoprotective autophagy which was suppressed by thymoquinone, resulting in a decrease in autophagy and an increase in apoptosis.
Conclusion: Autophagic cell death plays a crucial role in the fate of cells after temozolomide treatment, and mechanism of reduction of cell resistance to temozolomide by thymoquinone maybe attribute to blocking of autophagy.
Keywords: Temozolomide, Glioblastoma Multiforme, Thymoquinone, Apoptosis, Autophagy

Ps-41: Sustain Release of Insulin from Porous Scaffold for Cartilage Tissue Regeneration

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Objective: Cartilage defects are very difficult to heal due to its limited ability of self-repair and regeneration. Therefore cartilage tissue engineering using porous scaffolds, chondrocytes or human mesenchymal stem cell and bioactive instructive cues has been evolved promising approach to treat cartilage defects. Collagen as a natural biomaterial is extensively investigated for preparation of porous scaffolds for cartilage tissue engineering applications. Growth factor and therapeutics are employed for maintenance of cell viability, proliferation and promotion of tissue regeneration. Insulin administration has demonstrated its ability to prolong the survival of chondrocytes and prevent the formation of necrosis in 3D collagen hydrogel construct. Controlled and prolonged delivery of the insulin using PLGA micro beads into porous collagen matrix has been demonstrated to be useful in cartilage tissue engineering. Our purpose was to prepare a controlled insulin releasing scaffold with controlled pore structure as a bioactive 3D culture system for cartilage tissue engineering.
Materials and Methods: Insulin was microencapsulated in PLGA microbeads using w-o-w double emulsion technique. The recovered microbeads were washed with water and freeze-dried in a freeze drier. The collagen-microbead hybrid porous scaffold was prepared by a freeze-drying method using pre-prepared ice particulates of a diameter range of 150 μm-250 μm as porogen. collagen solution was mixed with the prepared microbead suspension at a ratio of 9:1 to prepare microbead dispersed collagen solution.The manipulation was carried out at 4ºC and the mixture was magnetically stirred.The final mixture was molded in a frame template. Then the mold was freeze-dried and cross-linked EDC and NHS in ethanol for 24 hours at room temperature.The cross-linked scaffold was washed and freeze-

dried to prepare hybrid scaffold of collagen-microbead. Bovine articular chondrocytes were cultured in tissue culture flasks DMEM containing 10% fetal bovine serum, 4500 mg/L glucose, 4 mM glutamine, 100 U/mL penicillin, 100µg/mL streptomycin, 0.1 mM nonessential amino acids, 0.4 mM proline, 1 mM sodium pyruvate and 50 µg/mL ascorbic acid. The confluent monolayer of the cells was harvested using trypsin/EDTA treatment and seeded into the scaffolds by dispensing 80 µL of cell suspension (7.5×10^5 cells/scaffold). The cell-scaffold constructs were incubated for 3 hours in a CO_2 incubator to allow the seeded cells to adhere over the scaffolds.

Results: The scaffolds had controlled pore structure and the large pores were replica of the ice particulates used during fabrication process. The large pores were connected to each other with interconnected pores. The hybrid scaffold exhibited a homogeneous spatial distribution of micro beads throughout the pore walls. The mechanical strength of the scaffolds was determined using a compression test. The result indicated the control and collagen-micro bead hybrid scaffolds had high mechanical strength (150,155KPa respectively).

The release profile from microbeads showed an initial burst release (33% in day 1) followed by a rise in cumulative insulin (upto 3 weeks) and a very slow release phase (4th week). The scaffolds showed high cell seeding efficiencies and the seeding efficiencies of the scaffolds were 87.12 ± 1.13% (control) and 86.99 ± 1.38% (experiment).

Conclusion: The collagen-micro bead hybrid scaffold demonstrated a high mechanical strength and a stable release of insulin for 4 weeks. The released insulin demonstrated its effect on cultured chondrocytes for their survival and proliferation. The bioactive hybrid scaffold should be useful for maintenance of prolonged survival and proliferation of cultured chondrocytes towards the application in cartilage tissue engineering.

Keywords: Insulin, Cartilage, Collagen, PLGA

Ps-42: Enhanced Hepatogenic Differentiation of Induce Pluripotent Stem Cells on Collagen-Coated Polyethersulfone Nanofibers

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Objective: Human-induced pluripotent stem cells (hiPSCs) as unlimited source with great differentiation potential have attracted many surgeons and scientists attention for cell replacement therapies and liver tissue engineering applications.

Materials and Methods: In the present study, Polyethersulfone (PES) nanofiberous scaffold was fabricated by electrospinning technique and their surfaces were modified by plasma treatment and collagen (COL) coating. Scaffold characterization was performed using scanning electron microscopy (SEM), ATR-FTIR and MTT assay. Then the hepatogenic potential capacity of hiPSCs after cultured on PES/COL scaffolds was evaluated using real-time RT-PCR and immunocytochemistry (ICC) during 20 days.

Results: SEM results showed nanofibrous and porous structure of fabricated PES/COL scaffolds before and after surface treatment with no significant effect on the scaffolds structure. Presence of collagen on the surface of scaffolds was confirmed using ATR-FTIR spectroscopy.

Results of MTT assay demonstrated that biocompatibility of scaffolds was significantly increased by collagen coating according to the enhanced proliferation rate of hiPSCs cultured on PES/COL in comparison to the PES. Analyses of two important definitive endoderm specific markers- SOX17 and FoxA2- using real-time RT-PCR and ICC indicated that these mRNA and protein levels were increased after 5 days of hepatogenic induction.

In addition, to determine hepatic differentiation of hiPSCs cultured on PES/COL, the expression of albumin and α-fetoprotein (αFP) were evaluated by ICC after 20 days while differentiated cells were intensely stained for albumin and αFP in comparison to the controls. Also Real Time RT-PCR analysis showed increased expression of albumin, αFP, cytokeratins 8/19 and Cyp7A1 genes over the course of the differentiation program.

Conclusion: Our results demonstrated that hepatogenic differentiation potential of hiPSCs were significantly increased by PES/COL nanofibrous scaffolds, suggestng that it could also be considered as a promising candidate for liver tissue engineering and regenerative medicine applications.

Keywords: hiPSCs, Hepatocyte, PES Nanofiber, Collagen, Tissue Engineering

Ps-43: Bioinformatic Evaluation of miR-548aa and rs13423759 in 3'UTR of ErbB4 as A Potential Prognostic Biomarker in Breast Cancer

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Objective: Breast cancer is the most common cause of cancer death among women worldwide. Aberration in signal transduction pathway of ErbB family in human tumors is a common phenomenon. ErbB4 as an oncogene and also tumor suppressor is one of the members of ErbB family. ErbB4 overexpression has been observed in approximately 50% of breast cancer cases.

miRNAs are the large subgroup of noncoding RNAs with 18-25 nucleotides inhibiting the expression of target genes by means of binding to their 3'UTR. They can also play role as an oncogene and/or tumor suppressor. In recent years, the association of some SNPs located in either miRNA seeds or 3'UTR of their target genes with the risk of breast cancer have been proved in some populations.
Materials and Methods: miRNASNP database was used to identify the miRNAs with the ability to bind to the 3'UTR of ErbB4 transcripts. In next step, miRTarBase and DAVID databases were used to investigate the function and the related signaling pathways of obtained miRNAs.
Results: In silico investigation of SNPs in the 3'UTR of ERBB4 gene showed that rs13423759 could alter the binding properties of miR-548aa. By reviewing the literature and with regards to molecular enrichment analysis from miRWalk and DAIVID database, we realized that miR548aa targetome could act in some molecular signaling pathways such as: PI3K-AKT, JAK/STAT and MAPK signaling pathways; therefore, miR-548aa could be considered as an oncomiRNA. Since SNP rs13423759 causes loss of binding between miR-548aa and its target (ERBB4), this SNP could be proposed as a good-prognostic factor by reducing the activity of miR-548aa.
Conclusion: SNP rs13423759 could function as a good prognostic factor by disrupting the repressive activity of miR548aa
***Keywords*:** Breast Cancer, ErbB4, microRNA, SNP

Ps-44: Surface Bio-Functionalization of Nanofibrous Scaffolds Using Cartilage Extracellular Matrix

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Objective: Tissue engineering approaches based on decellularized extracellular matrix (ECM) scaffolds have been widely used as an alternative strategy for organ reconstruction. In reality, The ECM is a structurally enviromental 3D complex, composed of the secreted products of resident cells which provides the mechanical framework for each tissue or organ and is a substrate for cell signaling. Here, we present ECM powders derived from human nasal septum cartilage to functionalize surface of nanofibrous scaffolds for cartilage tissue engineering applications.
Materials and Methods: Human cartilage was physically shattered, then decellularized sequentially with use of sodium dodecyl sulfate (SDS) and a nuclease solution and ground to powders by milling. Then electrospun composite scaffolds combining poly 3-hydroxybutyrate (PHB) and poly 3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) functionalized covalently with ECM powders using reactive carboxyl groups (-COOH) through alkaline hydrolysis. Moreover, human adipose-derived stem cells (hASCs) cultured on biofunctional and balk scaffolds as well as tissue culture plate during 21 days to investigate their metabolic activity.
Results: On histology, decellularized tissues showed most of the ECM components after removal of the cell fragments. In an *in vitro* study, cells were cultured on fibrous mats presenting these ECM powders showed a significant increase in metabolic activity comparing to untreated scaffold and tissue culture plates as control. Masson's trichrom staining also showed the preservation of the matrix structure integrity after decellularization.
Conclusion: We believe that human ECM powders could act as efficient biomaterial for tissue engineering and have great potential for meeting new challenges in regenerative medicine, particularly in relation to cartilage reconstruction.
***Keywords*:** Tissue Engineering, Scaffold, Cartilage, Decellularization

Ps-45: Effect Of Bosentan and Losartan on Oxidative Stress and Cortisol in Endothelin-1 and Angiotensin II Treated Rats

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Objective: The exact mechanism by which endothelin-1 (ET-1) and angiotensin II (Ang II), and their antagonist effects in physiology are controversial subjects among researchers. Therefore, the aims were to determine the effects of bosentan and losartan on oxidative stress and serum cortisol levels.
Materials and Methods: The design included two experiments. First, contain four groups: group 1 saline infusion, group 2 ET-1 infusion, group 3 Bosentan + ET-1, and group 4 Losartan + ET-1. Second one includes: Group 1 Saline infusion, group 2 Ang II infusion, group 3 Losartan + Ang II, and group 4 Bosentan + Ang II.
Results: In the experiment I, demonstrated that bolus infusion of losartan significantly decreased serum cortisol, while bosentan slightly reduced it versus ET-1. Beside that, from experiment II, bosentan could significantly decrease cortisol compared with Ang II neither losartan nor Ang II changed it significantly in comparison with Ang II and saline group. Furthermore, bosentan caused rising in MDA concentration according to ET-1 infusion, but losartan slightly decreased it. MDA in Ang II infusion dramatically became high in comparison with saline infusion, both losartan and bosen-

tan not significantly returned it to the base line levels. The serum glucose concentration clearly rose in losartan infusion, but bosentan affected it significantly. Serum chloride in both bosentan and losartan significantly increased according to ET-1. Both ET-1 and Ang II infusions for one hour led to increase Mg^{++} concentration in concomitant with saline infusion, while Bosentan and losartan did not change it as compared with ET-1 and Ang II respectively.

Conclusion: Both ET-1 and Ang II antagonists reduced cortisol levels, but they did not change lipid peroxidation marker as elevated by Ang II infusion. Interestingly, ET-1 and Ang II markedly can increase serum Mg^{++} levels, but their antagonists could not return to the normal levels.

Keywords: ET-1, Ang II, Cortisol, Magnesium

Ps-46: Disease Modeling of Systemic Sclerosis Disease through Induced Pluripotent Stem Cell Induction

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Objective: Systemic sclerosis is a connective tissue disease with unknown pathogenesis. A complex interaction among endothelial and immune cells results in fibroblast activation which produce high amount of extracellular matrix in damaged organs. Lack of suitable animal model or limitation of developmental studies in human, could direct us for development of new therapies. As we know, embryonic stem cells (ESCs) have unique characteristics in cell-based studies. However, availability of human ESCs is the major limitation of these cells. Induced pluripotent stem (iPS) cells may represent a potential strategy to overcome the limitations of human embryonic stem cells (ESCs) and have the potential to mimic human disease, and also can be differentiated into one or more cell types to find out diseases pathogenesis. Since the need for a reliable and reasonable source for scleroderma pathogenesis evaluation remains as a challenge, we decided to derive iPS from patients as an appropriate model to find out mechanism underlying this disease progression.

Materials and Methods: To achieve this, we obtained skin samples from three patients with SSC and three healthy volunteers as control. In the next step, we tried to establish several iPS cell lines from each patient using retrovirus vectors carrying the four defined pluripotency factors including Oct4(O), Sox2(S), c-Myc(M), and Klf4(K). Afterward, a series of experiments carried out to confirm their typical ES-like cellular characteristics with respect to pluripotency markers expression using RT-PCR and immunocytochemistry.

Results: Fibroblast derived iPS cells (SSC-hiPSC) were characterized and showed ESC like properties due to expression of OCT4, NANOG, TRA-1-60 and TRA-1-81 pluripotency markers using immunocytochemistry. It has been demonstrated that exogenous genes (OSKM) expression has been decreased dramatically in iPS cells against pluripotent genes (OCT4, NANOG). SSC-hiPSCs were able to differentiate to 3 germ layers *in vitro*. SSC-hiPSCs showed normal karyotype. These cells were also stained by alkaline phosphatase. To be mentioned, There was not any significant difference between normal and patients' derived iPS cells and also between different iPS cells from one or more patients due to their characterizations.

Conclusion: In this project, for the first time we were successful to derive iPS cells from human fibroblasts from systemic sclerosis patients, that can be used for differentiation to endothelial and fibroblast cells which may help us to find out pathogenesis mechanism of this disease.

Keywords: Systemic Sclerosis, Cell Therapy, Induced Pluriotent Stem Cells

Ps-47: Growth Properties and Differentiation Ability of Adipose Tissue Mesenchymal Stem Cells of Guinea Pig

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Objective: Mesenchymal stem cells (MSCs) are multipotent stem cells with capacity to differentiate into several mesenchymal lineages. MSCs can be isolated from a variety of tissues including bone marrow and adipose tissue, which are the most common sources of these cells. However, MSCs from these different sources have different characteristics. The aim of this study was the isolating of adipose tissue MSCs (AT-MSCs) from guinea pig and assessing their growth properties and differentiation potentials.

Materials and Methods: In this study, epididymal adipose tissues (ATs) were collected from 3 healthy young (4 months old) guinea pigs and the culture was expanded through eight successive passages. AT-MSCs at passages 2, 5 and 8 were seeded in a 24-well plate with 5×10^4 cells per well in triplicate. Cells were collected from each well 1-8 days after seeding and counted microscopically to determine population doubling time and cell growth curves. Furthermore, we analyzed AT-MSCs differentiation ability to osteogenic and adipogenic lineages by using alizarin red and oil red attaining

method.
Results: AT-MSCs attached to the culture flask and displayed spindle-shaped morphology in all passages. Growth curves showed good enough proliferation ability in all passages especially in the second and fifth passages. Growth and proliferation rate of AT-MSCs in the second passage was more than the fifth and eight passages. The PDT of the passages two, five and eight were 61.8, 64.2 and 73.8 h, respectively. According to the results, guinea pig AT-MSCs could easily differentiate into osteogenic and adipogenic lineages when cultured in specific culture media.
Conclusion: AT-MSCs were spindle-shaped in all passages. The increase in the PDT of AT-MSCs occurs with the increase in cell age. AT-MSCs from guinea pig can be an appropriate candidate for use in cell therapy and preclinical studies in guinea pig animal model.
Keywords: Mesenchymal Stem Cells, Guinea Pig, Adipose Tissue, Bone Marrow, Growth Properties

Ps-48: Isolation, Culture, Growth Kinetics and Osteogenic and Adipogenic Differentiations of Guinea Pigs Bone Marrow Derived Mesenchymal Stem Cell

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Objective: Mesenchymal stem cells (MSCs) from various species and sources can be enriched by isolation of cells with fibroblast-like morphology. They have a high ability to renew and differentiate into various lineages. MSCs have been studied in regenerative medicine because of their unique immunological characteristic. However, before clinical application, animal models are needed to demonstrate their safety and efficacy. The purpose of this study was isolating mesenchymal stem cells from guinea pigs bone marrow (BM) and assessing their growth kinetics.
Materials and Methods: Bone marrow tissues were collected from both tibia and femur bones of three healthy young (4 months old) Guinea pigs and cultured. The extracted cells were subcultured from primary to passage five. BM-MSCs of passages 2 and 5 were seeded in 24-well plates at a density of approximately 5×10^4 cells per well, in order to determine the population duplication time (PDT) and growth curves; each day, the cells of three wells were enumerated and the PDT was determined. Moreover, BM-MSCs of passage 3 were cultured in osteogenic and adipogenic differentiation media.
Results: BM-MSCs were adhered to the flask and showed a fibroblastic-like morphology when plated. Growth and proliferation rate of BM-MSCs in the second passage was more than the fifth passage except in day 6. The PDT of the passages two and five were 60.1 h and 65.6 h, respectively. Moreover, after culture of BM-MSCS in differentiation media, the cells differentiated toward osteoblasts and adipocytes were verified by positive staining with Alizarin Red S dye and Oil Red O staining, respectively.
Conclusion: Guinea pigs BM-MSCs were shown to be a valuable source of stem cells at passage 2 and they can be a good model in regenerative medicine and cellular studies.
Keywords: Bone Marrow, Mesenchymal Stem Cell, Guinea Pig, Characterization, Osteogenic and Adipogenic Differentiations

Ps-49: Fibrin Glue Derived of Cord Blood Plasma; An Experience of Royan Public Cord Blood Bank

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Objective: Routinely, Fibrin glue is produced from peripheral blood plasma. It contains fibrinogen, thrombin, factor 13 and calciumchloride. It is vastly used instead of sutures to seal and stop bleeding in surgeries. Fibrin glue is an approved agent by FDA that plays hemostatic role in body. Production of Fibrin glue from umbilical cord blood (UCB) as a biological waste makes this procedure safe and cost effective approach with therapeutic applications.
Materials and Methods: Fibrinogen was isolated from cord blood plasma using protamine sulphatare and by mechanical and chemical methods. Umbilical cord blood platelet (UCB-Pl) with concentration of 2×10^9 cells/ml (to provide coagulation factor 13) and calcium chloride were used to isolate and activate thrombin. In that stage, Fibrin clot was formed by mixing fibrinogen, thrombin and UCB-PL in defined and qualified concentrations. The prepared glue was used in different surgeries such as Pterygium, Cartilage avulsion, Crohn's disease, Kienbock as cell delivery vehicle.
Results: The results were acceptable by surgeons. Average clotting time was measured to be between 10-20 seconds and the adhesiveness has been stabilized for 1 month at room temperature and 37°C. The mean concentration of fibrinogen was 10.8 ± 2.3 mg/ml SD. The concentration of factor 13 in thrombin was 2.9 ± 0.4 IU/ml, PL 9.9 ± 2.8 and fibrinogen components 7.1 ± 0.9. Total protein concentration for fibrinogen component and thrombin are 16.6 ± 4.1 and 18.4mg/ml ± 3.5,

respectively.
Conclusion: Although the concentrations of fibrinogen and thrombin in cord Blood are less than peripheral blood, the clotting time is acceptable (in range of 10-20 seconds) and the stability is the same with commercial products.
Keywords: Fibrin Glue, Umbilical Cord Blood, Fibrinogen, Thrombin

Ps-50: Using Umbilical Cord blood Platelet Lysate: New Solution for Expansion of Cord Blood Mononuclear Cells with Therapeutic Application

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Objective: Expansion of cord blood mononuclear cells *in vitro* is a solution for the low number of umbilical cord blood unit cells in cord blood transplantation. Platelet extract has been introduced as a supplement for mesenchymal stem cell culture in cell therapy. The purpose of this study was to investigate the effect of umbilical cord blood platelet lysate (UCB-PL), in proliferation, survival and differentiation rate of mononuclear cells derived from cord blood.
Materials and Methods: Platelet lysate were prepared by mechanical method in concentration rang of 2×10^9 plt/ml from cord blood. Mononuclear cells were isolated by using hydroxyethyl starch from cord blood. They were seeded in 5×10^5-1×10^6 celles/ml and cultured in presence of Flt3L (50ng/ml), Tpo (10ng/ml), SCF (50ng/ml) concentrations of UCB-PL (0-10%) In the positive control group, cells were cultured in a medium containing cytokines while negative controls were cultured in a medium lacking cytokines. The effective dose and cell viability were assessed by Trypanblue. Colony assay method was used to assess the differentiation of mononuclear cells. The expression of $CD34^+$ as a stem cell marker was performed byqRT- PCR method.
Results: Higher concentrations of 5% UCB-PL was cytotoxic for cells. In the presence of 5% UCB-PL, MNCs had higher proliferation than other groups. In this particular group significant increase of colony forming units BFU-E, CFU-GMMM and CFU-GM were detected. An increased expression of $CD34^+$ gene for cultured MNCs in different days was also observed when compared to the other groups.
Conclusion: Our results indicate that 5% UCB -PL can be used as a supplement in MNC cultured cells and in cord blood hematopoietic stem cells for cell therapy purposes.
Keywords: Proliferation and Differentiation, Platelet Lysate Derived from Umbilical Cord Blood, Colony Assay

Ps-51: Differentiation of Alveolar Epithelial Type II Cells from mouse Embryonic Stem Cells by A549-Condition Medium Plus Hydrocortisone

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Objective: ESCs are pluripotent cells with the ability to differentiate *in vivo* and *in vitro* into all proper cell types of the embryo. Researchers found that a stepwise process -through the stages of lung embryonic development- is required to obtain efficient production of differentiated lung epithelium cells such as alveolar epithelial type II (AETII) cells. The aim of this study was to differentiate mESCs into AETII cells by using A549-Condition Medium (A549-C.M) plus hydrocortisone *in vitro*.
Materials and Methods: The mESC line, Royan 20, was induced to differentiate using adherent culture method and without the formation of embryoid body in serum-free differentiation (SFD) media. For definitive endoderm (DE) formation, IDE2 was added in the media for 6 days. For AETII-like cells induction, the resulting DE was treated with A549-C.M plus hydrocortisone in SFD media for 9 days. By the end of day 15, the produced cells were collected and analyzed by quantitative PCR, immunocytochemistry and flowcytometry for markers of embryo, DE and AETII cells. To confirm the generation of AETII-like cells on an ultrastructural level, TEM analysis was performed.
Results: Differentiated cells displayed increased SP-A, SP-B, SP-C and SP-D (surfactant proteins, AETII cells specific markers) expression consistent with AETII cells production. We founded ~24.12% of ESCs displayed immunoreactivity to SP-C (unique feature of AETII cells which is commonly used to identify these cells from other lung parenchymal cells) after exposure to A549-C.M plus hydrocortisone. There was minimal production of Foxa2 a marker of definitive endoderm and Oct4, a marker of pluripotency. TEM exhibited ultrastructural features characteristic of AETII cells, including cytoplasmic lamellar bodies (LB) -organelles that contain pulmonary surfactant proteins. Presence of the LB is a criterion traditionally used for the identifica-

tion of AETII cells.
Conclusion: We have developed a new *in vitro* model to differentiate AETII cells from mESCs for future basic research and potential therapeutic application.
Keywords: A549-C.M, Hydrocortisone, AETII Cells, mESCs, Differentiation

Ps-52: MicroRNAs Associated with Ground State Pluripotency Inhibit Differentiation

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Objective: MicroRNAs control the gene regulatory circuitry in embryonic stem cells (ESCs). Ground state pluripotency is a cell state in which pluripotency is established and maintained primarily via efficient inhibition of the ESCs' intrinsic propensity to differentiate. We hypothesized that microRNAs associated with ground state pluripotency might contribute to this differentiation-suppressing behavior.
Materials and Methods: Mouse ESCs were cultured and maintained under serum, 2i (dual inhibition of GSK3 and ERK signaling), and R2i (dual inhibition of TGFβ and ERK signaling) conditions, and subjected to small RNA sequencing. Overexpression of the candidate microRNAs was achieved by transient transfection of mature microRNA mimics. TaqMan and SYBR Green-based systems were used to quantify microRNAs and mRNAs, respectively.
Results: MicroRNA profiles showed that 2i- and R2i ESCs (which are collectively considered here as ground state ESCs) were much more similar to each other than to serum-grown cells. Chromosomes 2 and 12 were observed to contribute the highest number of microRNAs to the ESC microRNAome in serum- and ground-state ESCs, respectively. A closer look at these two chromosomes revealed that a large microRNA cluster embedded in the Sfmbt2 locus was upregulated in serum ESCs while another large microRNA cluster in Dlk1-Dio3/Meg3 locus was upregulated in ground state ESCs. Because microRNAs upregulated in ground state ESCs were predicted to inhibit developmental pathways, we performed functional analysis for the candidate microRNAs associated with ground state pluripotency and observed that they inhibited multi-lineage differentiation of ESCs both generally and efficiently.
Conclusion: We found that the expression pattern of large microRNA clusters embedded in imprinted loci of the genome can distinguish serum- from ground-state ESCs. Additionally, we identified novel microRNAs in 2i- and R2i-ESCs contributing to the maintenance of ground state pluripotency, generally by blocking ESC differentiation.
Keywords: MicroRNA, Ground State Pluripotency, Embryonic Stem Cells, Differentiation

Ps-53: The Fate of Leukemic Stem Cells in The Hands of Musashi and Numb

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Objective: Deregulation of key genes that control self-renewal and cell fate decisions in hematopoietic stem cells (HSCs) can contribute to the onset and progression of leukemia. Musashi2 (Msi2) is a RNA-binding protein that is highly expressed in HSCs and leukemic stem cells (LSCs). The elevated level of Msi2 leads to the downregulation of the cell-fate determinant, Numb, which is in association with other signaling pathways such as hedgehog and notch that ultimately reduces the growth of LSCs. In this study we knocked down Msi2 using siRNA. To investigated the effect of Msi2 knockdown on the expression levels of Numb, P21 (the cell cycle gene) and Bcl-2 (an anti-apoptosis gene), we used HL-60 and K562 cell lines (an AML and CML cell line, respectively).
Materials and Methods: Lipofectamin RNAiMax was used for transfection of siRNA. For real-time PCR we used SYBR-Green master mix purchased from Takara (Japan). Lipofectamin RNAiMax, siRNA and other reagents were purchased from Invitrogen.
Results: Real-time PCR analyses showed high expression of Msi2 in HL-60 and K562 cells, while with inhibition of Msi2 by siRNA, Numb is increased. Msi2 silencing inhibited cell growth and caused cell cycle arrest by increasing the expression of p21. In addition, knocking-down of Msi2 promoted cellular apoptosis via the downregulation of Bcl-2 expression.
Conclusion: Commonly used drugs for leukemia, such as Imatinib which targets tyrosine kinases, do not effectively eradicate LSCs which are thought to be responsible for the leukemia progression. Therefore, identification and inhibition of signaling molecules/pathways such as Musashi-Numb axis that involved in the survival and self-renewal of LSCs will offer effective therapeutic strategies aiming to eradicate LSCs. Musashi is a master switch ("on" and "off" key) for Numb. Musashi-Numb pathway can control the differentiation and apoptosis of LSCs, and raise the possibility that targeting this pathway may provide a new strategy for the therapy of LSCs.
Keywords: Musashi, Msi2, Numb, CML Stem Cells

Ps-54: Effects of Microtubules Network on Response of a Mesenchymal Stem Cell to Fluid Flow: A Mechanical Modulation Study

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Objective: Investigating effects of mechanical stimulations on stem cell fate has been carried out widely and is currently one of the developing fields in stem cell related researches. One of the well-known aspects of these biophysical cues is the effect of dynamic characteristics of fluid flow on stem cell behavior at a specific condition. Current study is going to investigate the mechanical response of a mesenchymal stem cell (MSC) as a whole, and subcellular elements such as nucleus and microtubules individually, located at a typical parallel plate flow (PPF) bioreactor with the aid of finite element method (FEM).
Materials and Methods: Two different FEM models of MSC were employed to simulate this phenomenon. The first one proposed to be an ideal ellipsoidal bulk body without any subcellular elements and the other proposed to have an interconnected network of microtubules and nucleus as well. These models were located at the fully developed section of a PPF bioreactor and experienced laminar flow regimes at the biological range. Using the fluid-structure interaction method, the solutions for both solid and fluid domains were obtained at once. Further to take the elements to be linear elastic material, the problem was solved at stationary state.
Results: The results showed that the hydrodynamic pressure rather than the shear rate is the dominant factor in fluid flow induced deformation of the MSCs. In addition, the notable effects of subcellular elements on the mechanical properties and response of the cell were observed. Also the most affected regions and elements under such circumstances and withstanding of microtubules under stresses exerted to the cell were acquired.
Conclusion: Involving effective subcellular elements besides the logical simplifications can elucidate the response of the MSC to mechanical stimulations. Furthermore, this kind of simulations can help researchers to optimize designing variables of the cellular experiments *in vitro*
Keywords: Mesenchymal Stem Cell, Microtubules, Mechanical Stimulation, Fluid-Structure Interaction, FEM

Ps-55: Electrospun PCL/ Collagen Fibrous Scaffold Modified with Nanographene for Application in Tissue Engineering

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Objective: Tissue engineering as a subfield of regeneration medicine is based on living cells, signaling factors, scaffolds and bioreactors. Scaffolds which are substrates for cell attachment, can act as physical and chemical signaling factors, simultaneously. In this study, polycaprolacton/collagen nanofiber modified with graphene nanoplatelet was utilized to prepare a 2D scaffold using electrospinning technique. Nanofibers are used to mimic the extracellular matrix conditions. On the other hand, graphene is able to absorb proteins and low molecular weight chemical molecules on its surface and consequently affect cell proliferation and differentiation. Composites comprising nanofibers and graphene can be used as new scaffolds in tissue engineering. In this study, SEM, FTIR and MTT analyses were used for characterization and cell compatibility assessment of scaffold.
Materials and Methods: Nanofibrous PCL/ collagen scaffolds were prepared using electrospinning technique. PCL/ collagen (75:25) was dissolved in 90% acetic acid to obtain a homogeneous 15% (w/v) solution. The electrical field used for electrospinning, flow rate and distance between tip and collector were 20 kV, 0.3 ml/h and 15 cm, respectively. Dispersed 1% (w/w) nanographene in DMF was sprayed on the nanofiber. SEM was used to study the morphology of prepared scaffold.
Scaffold was cross-linked utilizing glutaraldehyde vapor with a concentration of 10% for 64 h. Before cell culture, the prepared scaffold was exposed to ultraviolet (UV) light for 45 min on both sides, in order to be sterilized, and then placed in a 24-well culture plate. The effect of scaffold on the proliferation of rat adipose mesenchymal stem cells (rAMSCs), obtained from national cell bank of Iran, was studied by incubating cells with scaffold extract as culture medium for 24 h and then performing MTT test. Cell attachment on the scaffold was examined by SEM.
Results: SEM image of scaffold indicated the modification of nanofibers with nanographene. In FTIR diagram, 1750-1640, 3500-3100, 1350-1000 and 2750-1750 peaks were related to C O, N H, C N and graphene, respectively, confirming the preservation of material chemical structure. SEM images revealed the excellent cell attachment on the scaffold. Results of MTT assay showed 22% increase in cell proliferation compared to the control, during 24 h.
Conclusion: The results of scaffold examination initially approved its application in tissue engineering. The effect of nanofibers and graphene in neural tissue engineering was shown. Graphene causes the neuritis outgrowth from the neuron cell. Therefore, this scaffold can be a good substrate for neural differentiation of stem cells.
Keywords: PCL/ Collagen Nanofibers, Nanograghene, Stem Cell, Tissue Engineering

Ps-56: The Effect of Different Concentrations of Neuronal Conditional Medium (NCM) on Differentiation of Wharton's Jelly Mesenchymal Stem Cells to Insulin Producing Cells by Assessment of Nestin and Pdx1 Expression

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Objective: Today, type I diabetes prevalence falls into an increasing rate, so the various approaches are examined for its control and treatment. Among them cell therapy, as a new method, come into consideration. But still finding an appropriate source of stem cells and their differentiation to insulin producing cells (IPCs) need more investigations. In the present study, we examined the effect of neuronal conditional medium (NCM) on differentiation of human Wharton's jelly mesencymal cells (hWJMCs) by evaluation of Pdx1 and Nestin expression.
Materials and Methods: The hWJMCs were isolated by explant culture and they were assessed with flowcytometery for expression of stem cell surface factors including CD34,CD44,CD45, CD73 and CD90. NCM medium was harvested from Wistar rats brain extract. Then the cells were cultured in various concentrations of NCM medium (20, 40 and 60%) supplemented with 2% FBS in DMEMF12 (5 mM glucose) + 10 mM nicotinamide and 1/100 ITS (Insulin-transferrin-selenium, Gibco) for one week. After that, the expression of Nestin and Pdx1 were evaluated by immunocytometery and semi-quantitative RT-PCR, respectively.
Results: The isolated cells demonstrated the mesenchymal nature, because they were negative for CD34 and CD45 whereas they expressed CD45, CD73 and CD90. Also, semi-quantitative RT-PCR showed that higher NCM concentrations could lead to more insulin and Pdx1 expression which are key factors in differentiation to IPCs.
Conclusion: Some studies demonstrated that through the IPCs differentiation, the selection of Nestin-positive cells can lead to better beta cells differentiation. On the other hands, in our study NCM, as an inducer for Nestin expression during differentiation to insulin producing cells could lead to more Nestin- and Pdx1-positive cells, so that more NCM concentrations could lead to higher Nestin and Pdx1 expressions. therefore it can lead to better IPCs differentiation.
Keywords: Wharton's Jelly, Nestin, Pdx1, Insulin

Ps-57: The Effect of Co-Culture Pancreatic Islets on Differentiation of Mouse Embryonic Stem Cells into Insulin Producing Cells

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Objective: In this study mous embryonic stem cells (mESCs) were indirectly co-cultured with pancreatic islets for 14 days. Embryonic stem (ES) cells have a pluripotent ability to differentiate into a variety of cell lineages *in vitro*. The aim of the present study was to investigate the roles of the co culture of mESc with pancreatic cells in the differentiation of insulin-producing cells from mES cells treatment of mouse with diabetes mellitus. We present here a method for forming immature islet-like clusters of insulin-producing cells derived from mES cells. We developed a co-culture system of mouse embryoid bodies (EBs) and pancreatic islets cells.
Materials and Methods: Before differentiation, EBs formed by a 5 day hanging drop culture. Pancreatic islets were isolated from overnight-fasted male NMRI mice by Lacy and Kostianovsky modified collagenase digestion method and islets were tested for their specificity by dithizone (DTZ) staining. The resulting cells were analysed for pancreatic gene and protein expression by immunocytochemistry and qRT-PCR.
Results: The differentiated cells derived from method of morphology were represented as pancreatic beta cells. Expression of beta specific markers including Sox17, FoxA2, Pdx1, Ngn3 and insulin were confimed by qRT-PCR and immunofluorescenc.
Conclusion: The results of this study indicated that mouse embryonic stem cells could differentiate into insulin-producing cells in presence of pancreatic extract. Therefore, application of these results facilitates *in vitro* production of beta cells from stem cells.
Keywords: Mouse Embryonic Stem Cell, Differentiation, Co-Culture, Pancreatic Islets, Insulin-Producing Cell

Ps-58: Novel Hybrid ECM/Chitosan Scaffold in Cartilage Tissue Engineering Application

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Objective: Articular cartilage has very little capacity for spontaneous healing due to tissue's avascular nature. Although many repair techniques have been proposed over the past decades, none has successfully regenerated long-lasting tissue to replace damaged cartilage. Tissue engineering have recently demonstrated tremendous approaches for regeneration of cartilage tissue lesions. The aim of this study is to construct 3D biodegradable scaffolds which enhance cell viability and proliferation. Chitosan's biocompatibility, biodegradability and similarity to glycosaminoglaycans supports chondrogenic activities and makes it desirable for cartilage tissue engineering. We hypothesized that the incorporation of ECM in chitosan matrix can enhance cell attachment and proliferation as a result of providing informational signals and unique composition.
Materials and Methods: In this study, extra cellular matrix (ECM) was obtained by decellularization process of bovine cartilage. Novel chemically cross-linked hybrid scaffolds were fabricated by freeze-drying technique. Influence of chitosan amount on structural and mechanical properties of scaffolds with different concentration of chitosan 4, 5 and 6% was evaluated by SEM and compression test. This investigation were also applied on scaffolds with 1 and 2% ECM, simultaneously. For showing the effect of ECM presence on scaffold's biocompatibility and biodegradability, scaffolds with 1, 2 and 3% ECM were constructed and properties was evaluated by MTT assay and enzymatic biodegradation.
Results: The results demonstrated that the scaffold with 5% chitosan showed best mechanical and structural properties. The insignificant impact of ECM concentration on mechanical and structural scaffold's properties is also proven. The concentration of 2% for ECM shows the best results for cell viability and scaffold swelling and biodegradation.
Conclusion: By considering the Conclusion which are taken from the result of SEM, biodegradability tests, MTT assay and swelling ratio, the scaffold with 5% chitosan and 2% ECM was shown to have accepted chondrocyte viability. Our results suggest that hybrid novel scaffold of chitosan/ECM could have potential use in cartilage tissue engineering.
Keywords: Cartilage Tissue Engineering, Extra Cellular Matrix (ECM), Chitosan, Decellularization, Hybrid Scaffold

Ps-59: Remyelination following Transplantation of Oligoprogenitor Cells Derived from Bone Marrow Stromal Cells into Demyelination Model

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Objective: Bone marrow stromal cells (BMSCs) are multipotent stem cells that can differentiate into various cell types. Here, we transplanted oligoprogenitor cells (OPC) derived from BMSC into rat demyelination model to evaluate their myelinogenic activity.
Materials and Methods: BMSCs were differentiated to OPC via glial inducers and then were grafted in sito demyelinated corpus callosum. The myelin formation changes were investigated using histological and immunocytochemistry techniques.
Results: Our results indicated myelin regeneration after transplantation of OPC into the demyelinated corpus callosum.
Conclusion: Transplantation of OPC derived BMSC can be usable, as a desirable source for treatment strategies, for demyelination diseases such as multiple Sclerosis (MS).
Keywords: Bone Marrow Stromal Cells, Demyelination, Model,Transplantation

Ps-60: Generation and Maintenance of Expandable Motor Neuron Progenitor Derived from OLIG2-GFP Knock-In Human Embryonic Stem Cells

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Objective: The efficient derivation of (OLIG2+) motor neurons progenitor from embryonic stem cells is a sought-after goal in the understanding, and potential treatment, of motor neuron diseases. Conditions that promote the robust generation of motor neuron progenitors from embryonic stem cells and that promote the survival of differentiated motor neurons ex vivo are likely, therefore, to be critical in future biological/ therapeutic/ screening approaches.
Materials and Methods: In this study, we describe a gene-targeting protocol to knock-in a GFP cassette into OLIG2 gene loci in human pluripotent stem cells (hPSCs), and then use the genetically tagged hPSCs to guide *in vitro* differentiation, immunocytochemical and electrophysiological profilings. The Olig transcription factors have key roles in the transcription regulatory pathways for the genesis of motor neurons (MNs) and oligodendrocytes (OLs).
Results: We have used a four stage protocol to gener-

ate spinal motor neurons (MNs) from human embryonic stem cells (ESCs). To optimize the differentiation protocol reporter lines marking the NSC and MNP stages were used. Additionally, we find that OLIG2-positive cells at the MNP stage can be cryopreserved and then recovered to continue the process of MN differentiation, thereby providing a highly stable and reproducible technique for bulk differentiation. MNPs were differentiated to MNs expressing the marker HB9, demonstrating that mature-MNs can be generated with this protocol.
Conclusion: These results indicate that OLIG2-GFP labeled MNPs will facilitate basic and translational studies using human motor neurons at a minimal cost.
Keywords: Motor Neurons Progenitor, OLIG2-GFP, Knock-In

Ps-61: Three-Layered Alginate Capsules for Pancreatic Islet Microencapsulation

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Objective: Diabetes is one of the most prevalent and destructive diseases around the world that is the sixth leading cause of death in the United States. One of the promising treatments for type 1 diabetes is Islet transplantation. In a case of allotransplantation, patients require life-long immunosuppression to overcome the rejection of transplanted islet cells. Considering that anti-rejection drugs can cause side-effects, the incorporation of alginate encapsulation technology is one of the strategies to replace life-long immunosuppression regimen. However, there are some obstacles for the clinical application of encapsulated islets such as fibrosis formation during transplantation, and less vascularization which can cause failure of encapsulated islets. Heparin-mimicking molecules have been shown to have anti-fibrosis properties *in vivo*, allowing to induce local neovascularization. Therefore, in this study we used Sulfated alginate as a thick outer coating of the encapsulated islets.
Materials and Methods: To produce the three-layered capsules, 2% alginate solution were sprayed using an air-driven encapsulator. For the 2nd coating layer, 0.2 Poly-l-lysine (PLL) were used. Then, the capsules were coated with the sulfated alginate. For characterization of the three-layered [alginate-PLL-sulfated alginate (APSA)] microcapsules, the 3rd coating layer was performed using the FITC conjugated alginate and imaged by fluorescent microscopy. To investigate the *in vivo* properties of the microcapsules, they were transplanted under the kidney capsule of the mice and eight weeks later kidneys were explanted, paraffin embedded, sectioned and analyzed for fibrosis formation and vascularization using trichrome and H&E staining, respectively.
Results: Fluorescent imaging showed intact layer prior to transplantation. Grafts were removed after eight weeks, APSA microcapsules showed significantly higher levels of neovascularization compared to the control group [microcapsules lacking sulfated alginate (APA)].
Conclusion: Microcapsules containing sulfated alginate, as their outer layer, can be used in islet transplantation technology.
Keywords: Type 1 Diabetes, Pancreatic Islets, Encapsulation, Sulfated Alginate

Ps-62: The Evaluation of Hematopoietic Stem Cells Potential after 2 Years Cryopreservation

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Objective: Evaluation of number, potential and functional capacity of cord blood stem cells after long term storage is essential for their therapeutic uses in hematological malignancies and non-malignancies. Therefore, this study was aimed to assess the quality of cord blood units stored in Royan public cord blood bank, before and after cryopreservation
Materials and Methods: To check the quality of cryopreserved cord blood units, the total number of cells, percentage of viable cells, colony-forming ability, the number of $CD34^+$ cells before and after freezing was examined on 50 samples.
Results: Our results determined that the mean value of total nucleated cells in 50 cord blood samples was $7.6\times10^8 \pm 1.12\times10^8$. The number and viability of mononuclear cells as well as colony forming potential significantly decreased after cryopreservation; however the count of viable $CD34^+$cells did not show significant changes post thawing. There was no significant correlation between the numbers of nucleated cells and $CD34^+$ cells.
Conclusion: Increasing storage time significantly reduces the number of nucleated cells and differentiation potential of hematopoietic stem cells, but not the number of $CD34^+$ cells. Improvement of cryopreservation techniques are necessary to maintain the function of cord blood stem cells.
Keywords: Hematopoietic Stem Cells, Cryopreservation, Colony Forming Units Assay

Ps-63: Human Embryonic Stem Cell-Derived Insulin-Producing Cells Maintain Viability and Function after Alginate Encapsulation

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Objective: Type 1 Diabetes is an autoimmune disease where the body's immune system destroys the specialized insulin secreting cells in the Islets of Langerhans, called βeta cells. In the absence of insulin, blood sugar levels rise because insulin is the primary hormone that regulates glucose uptake in muscle and adipose cells. Islet allotransplantation involves the transplantation of islets harvested from human pancreas donors into diabetic recipients. One of the biggest limitations of this procedure is the need for long-term immunosuppression. Islet microencapsulation is a technique, which can protect the islets within biocompatible microscale devices before transplantation. Encapsulating islets within permeable hydrogels such as alginate provides an effective immune barrier against host immune attack. In this study, we demonstrate that the human embryonic stem cells derived-insulin-producing cells (hESC-IPCs) remain viable and functional after alginate encapsulation.
Materials and Methods: The hESC-IPCs were derived using proprietary *in vitro* protocols at Novo Nordisk (Denmark) through an ongoing collaboration. The hESC-IPCs were shipped to our laboratory at Irvine, CA and then analyzed for viability and function. The percentage of viability was analyzed using propidium iodide (dead) and calcein AM (live), quantified with a microplate reader and imaged using a Nikon TIE (10x, 20x). The hESC-IPC function was analyzed by using a glucose-stimulated insulin response (GSIR) where the cells were incubated for one hour in low glucose (2.8 mM), high glucose (28 mM), high glucose + IBMX (28 mM +2 μM IBMX). The stimulation index (insulin release High+/Insulin release in Low) and the maximum stimulation index (Insulin release in High+/Insulin release in High) were calculated. The GSIR assay was performed in triplicate. The hESC-IPCs were encapsulated in 2.5% Ultra-Pure Low Viscosity Mannuronate Alginate (NovaMatrix® PRONOVA™ UP LVM) alginate solution. They were then incubated at 37ºC, 5% CO_2 overnight following which their viability and insulin release characteristics were analyzed as described above. All data reported at mean ± SEM. Statistical analysis was performed using a paired student t-test, and a $P<0.05$ was deemed statistically significant.
Results: The hESC-IPCs pre-encapsulation were shown to be 84 ± 2% viable and the stimulation index (SI) was 2.3 ± 0.1 (n=3, in triplicate). After alginate encapsulation (Nisco Inc. 9v, 3-4psi), the hESC-IPCs were 81 ± 1% viable and the insulin secretion was evaluated using a GSIR assay. The calculated SI increased to 2.6 ± 0.3 after encapsulation. The data showed that after encapsulation the hESC-IPCs did not show a significant change in viability ($P=0.2$) or function ($P=0.06$). This study demonstrates that hESC-IPC viability and function is not adversely impacted following alginate encapsulation.
Conclusion: The ability to produce functional viable stem cells would represent a significant advancement in the treatment of diabetes via transplantation of insulin-producing cells. Being able to protect the cells in a semipermeable membrane would permit transplantation without the need for chronic immunosuppression. In this study we demonstrated that hESC-IPCs maintained membrane viability and function after alginate encapsulation. Current studies including cellular characterization of hESC-IPCs using flow cytometry on both the free and alginate encapsulated hESC-IPCs are underway and the expression of insulin, glucagon and relevant stem cell markers is being evaluated. *In vivo* studies are being performed to evaluate alginate encapsulated hESC-IPC function in diabetic mouse models to identify the minimal dose required to reverse experimental hyperglycemia.
Keywords: hESC, Isulin, Diabetes, Encapsulation

Ps-64: Direct Reprogramming of Human BMSCS into Functional Renal Cells

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Objective: Human bone marrow stromal cells (BMSCs), also known as bone marrow-derived mesenchymal cells, are gaining recognition in regenerative medicine applications. While the majority of investigations has focused on reprogramming somatic cells into pluripotent stem cells, there is a paucity of studies on the reverse reprogramming of BMSCs towards the aquisition of a somatic cell phenotype. We inquired whether BMSCs could be reverse-reprogrammed to acquire additional desired properties of somatic cells.
Materials and Methods: Streptolysin-O reversibly permeabilized BMSCs were exposed to renal proximal

tubular epithelial -immortalized or primary- cell-free extract and their reprogramming process was evaluated using *in vitro*, *ex vivo* and *in vivo* assays.
Results: Human reveribly permeabilized BMSCs exposed to renal cell extract, underwent morphological changes: formation of "domes" and tubule-like structures, and acquired epithelial functional properties as transepithelial resistance, albumin binding and uptake and specific markers such as E-cadherin and Aquaporin 1. Transmission electron microscopy revealed the presence of brush border microvilli and tight intercellular contacts. RNA sequencing showed tubular epithelial transcript abundance and revealed the upregulation of components along the EGFR pathway. Reprogrammed BMSCs integrated into self-forming kidney tissue and formed tubular structures. Infusion of reprogrammed BMSCs in immunodeficient mice with cisplatin-induced acute kidney injury engrafted into proximal tubuli, reduced renal injury and improved function. Single cell derived clones retained the molecular and functional characteristics of the reprogrammed BMSCs throughout passages.
Conclusion: Thus, the direct reprogramming of human BMSCs may pave the way for future therapeutic applications.
Keywords: Direct Reprogramming, BMSCs, Renal Proximal Tubular Epithelial Cells, Cell-Extracts, Cell Therapy

Ps-65: The Effect of Chemotherapy Drugs on Pluripotent Stem Cell Markers in SKOV-3 Cancer Cell Line

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Objective: Different Researches have demonstrated that cancer is originated from a subpopulation of tumor cells with stem cell characteristics named cancer stem cells (CSCs). Nanog and Oct4 as pluripotent markers of stem cells are involved in promoting tumorigenesis through regulation of CSC population. The aim of this study was to examine effect of chemotherapy drug on SKOV-3 cancer cell line through apoptotic and pluripotent gene deregulation.
Materials and Methods: The human epithelial ovarian cancer cell line, SKOV-3 was cultured as monolayer in 25 cm^2 flask in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The numbers of live cells were calculated with trypan blue staining method using hemocytometer, and seeded into 96-well flat-bottomed culture plates. The cells were treated with Paclitaxel, Silibilin and Paclitaxel plus Silibilin and also one considered as control without treatment. MTT assay was carried out to determine cell viability. Following obtaining IC50, drug appropriate concentrations were applied to investigate the expression of apoptotic genes including *P21* and *P53* and pluripotent genes in SKoV3 cancer cell line using real-time PCR.
Results: Cell growth was inhibited considerably ($P<0.05$) by combination of Paclitaxel and Silibilin compared to the other groups after 48 hours of treatment. Also, investigated genes were dysregulated in treated groups especially in combination treatment. P53 and P21 showed over-expression in treated cells.
Conclusion: Our results showed that combination chemotherapy drugs may affect cancer stem cells more efficient than other ones. The increased level of P53 and P21 which are the tumor suppressors, may act against the self-renewal of stem cells and causes down regulation of pluripotent markers in cancer cells. We will further analyze transcriptional regulation, the Nanog signaling cascade, upstream regulators and downstream effectors of the *Nanog* and *Oct4* in treated cancer cell line.
Keywords: Chemotherapy Drugs, Pluripotent Stem Cell Markers, SKOV-3

Ps-66: Effects of Thymoquinone on Glioblastoma Cell Line

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Objective: Glioblastoma multiforme is the most common tumor of the central nervous system and its standard care includes surgical resection, radiotherapy and chemotherapy with temozolomide. Thymoquinone, the main active constituent of the crude extracts of the seeds of Nigella sativa Linn, has been shown to have potent antitumor activity in a variety of cancers. Treatment of glioblastoma multiforme with current standard methods, is problematic because of its recurrence and resistance to this drug. We therefore investigated the effects of thymoquinone on human glioblastoma cell line.
Materials and Methods: U87MG cells were cultured in DMEM/F12 supplemented with 10% FBS without antibiotics at 37°C and 5% CO_2 in a humidified incubator. Cells were treated with 10, 20, 50, 100, 150 and 200 µM thymoquinone for 24, 48, 72 and 96 hours and viability was determined using MTT assay. The concentration of thymoquinone that induced 50% cell inhibition against U87MG cells (IC50) were calculated by GraphPad Prism 5 software. Nitric oxide concentration was measured by Griess reagent. Cell death was analyzed by Acridine Orange –Ethidium Bromide double

staining and TUNEL assay. Data were analyzed by one-way ANOVA and P<0.05 was considered significant.
Results: The results showed that thymoquinone decreased significantly cell viability in U87MG cells, in a concentration and time dependent manner. Thymoquinone significantly decreased glioblastoma cells nitric oxide secretion. After treatment with thymoquinone, the number of apoptotic cells were increased. Necrosis was negligible.
Conclusion: Thmoquinone can provide an alternative therapeutic approach for the treatment of glioblastoma.
Keywords: Glioblastoma Multiforme, Thymoquinone, Nitric Oxide, Apoptosis

Ps-67: Purmorphamin Small Molecule Enhances The Expression of Motor Neuron Markers in Endometrial Derived Neurons

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Objective: Purmorphamin (PMA) is the agonist for a 7-pass protein in shh signaling pathway, called smoothened. This small molecule causes neural differentiation progressing. The differentiated cells express specific markers or proteins that shows the cell identity. NF, Chat and B-tubulin are the specific proteins of motor neurons. Expression of these markers during cell differentiation shows that the cells have become motor neurons. The purpose of this study is to investigate the expression of neural specific markers on motor neurons differentiated by PMA.
Materials and Methods: In this study endometrial stem cells were enzymatically extracted from endometrial tissue. After third passage, the flow cytometry was done for CD90, CD105 and CD146. For differentiation, the cells were treated with differentiating medium containing B27, FGF2, IBMX, 2ME and PMA for 18days. Then immunocytochemistry analysis was done for neural markers including Tuj-1, NF (neurofilament) and Chat markers.
Results: Flow cytometry analysis showed that hEnSCs were positive for CD90, CD105 and CD146. The immunocytochemistry results showed that cells expressed Tuj-1, NF and Chat markers.
Conclusion: Expression of neural specific markers in the endometrial derived motor neurons that are differentiated by the small molecule PMA suggest that we can utilize various small molecules in differentiation of neurons to use in cell therapy.
Keywords: Endometrial Stem Cells, Motor Neuron, Differentiation, Small Molecule

Ps-68: Effects of Cell Spreading and Its Implications on Estimating Nucleus Deformations and Stresses Using Finite Element Method

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Objective: Several attempts have been made to estimate stress field inside various cells in different physiological or experimental situations using finite element method (FEM). These attempts simulate the active contraction either using pressure -which surrounds cell's periphery- or a thermal analogy -which cools the cell to a certain temperature under a reference temperature. In this research, we consider the first and the final approximated geometry of a cell and apply a prescribed deformation which converts initial state to final state. Then we obtain the nucleus deformation and stresses. Finally we compare the range of generated force on the cell substrate and nucleus with the available force range data.
Materials and Methods: For the mechanical properties of a mesenchymal stem cell, we use the data available in literatures in which the mechanical properties of cell cytoplasm at the initial state is suggested to be 400 kPa and at the final state to be 1000 kPa. We model the cell as a viscoelastic material and use the shear modulus and relaxation time reported to be 400/((4)(1.4)) Pa and 94.81s , respectively. Since the cell undergoes strain stiffening during spreading we express the Young's modulus of the cell as a linear function of time. Finally we use a finite element model of the cell for spreading simulation.
Results: The average stresses generated on the projected area of the cell depending on its spreading varies from 3000 Pa to 8000 Pa for 1000 μm^2 to 2000 μm^2 spreading area, respectively. This is comparable with the traction stresses reported by traction force microscopy.
Conclusion: Using many developed imaging technologies such as confocal imaging we can capture the shape of cell during spreading and convert the initial state to the desired state and find an approximation for stress field on cell body and nucleus.
Keywords: Cell Spreading, Finite Element Method Strain Stiffening, Mesenchymal Stem Cells, Nuclear Deformation

Ps-69: Roles of Long Noncoding RNAs in Reprogramming of Cells

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Objective: Long non-coding RNAs (lncRNA) are a type of non-coding RNAs (ncRNAs) that recently have emerged as an important class of regulators of gene expression. lncRNAs exert critical functions in adult tissue stem cells, embryonic stem cell (ESCs) and induced pluripotent stem cells (IPS Cells). Scientists characterized the transcriptional reorganization of lncRNAs that occur upon derivation of human iPSCs and identify numerous lncRNAs whose expression is linked to pluripotency. we review recent developments and studies that illuminate the roles of lncRNAs in reprogramming and stem cell biology.
Materials and Methods: In order to compile this paper, we used highly cited articles with keywords including Long non-coding RNAs (lncRNA) , stem cells, and reprogramming presented in credible databases PubMed and Embase from 2006 to 2014.
Results:
Conclusion: Analyzing single-cell transcriptomes during somatic cell reprogramming, characterize hundreds of long non coding RNAs that are dynamically expressed at defined stages of reprogramming. lncRNAs contribute to the pluripotent state and can be used to assess reprogramming status. Cellular reprogramming highlights the epigenetic plasticity of the somatic cell state. Long non-coding RNAs (lncRNAs) have emerging roles in epigenetic regulation, but their potential functions in reprogramming cell fate have been largely unexplored.
Keywords: Long Non-Coding RNAs (lncRNA) , Stem Cells, Reprogramming

Ps-70: A Simple and Effective Method for Differentiation of Fat- Derived Mesanchymal Stem Cells into Insulin Producing Cells

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Objective: Type 1 diabetes (T1D) is a disease that involves the destruction of pancreatic insulin producing β-cells. Currently, there is no definitive cure for diabetes. Since mesanchymal stem cells (MSCs) have immunomodulatory properties and can differentiate toward the insulin producing cells (IPCs), we investigate the influence of beta-mercaptoethanol (beta-ME) in inducing the differentiation of human fat derived MSCs into insulin-secreting cells.
Materials and Methods: Adipose tissue-derived MSCs were isolated from liposuction. After characterization, stem cells differentiation was induced in two stages; low glucose-DMEM supplemented with 20% FBS, beta-ME (1 mmol/l) and nicotine amide (10 mmol/l) in pre-conditioning stage, followed by glucose-DMEM and nicotine amide (10 mmol/l) in the second stage.
Results: The differentiated cells have characteristics of IPCs including islet-like clusters, spherical, grape-like morphology, insulin secretion, positive for dithizone, glucose stimulation and expression of pancreatic endocrine cell marker genes including PAX4, Glut2, PDX-1 and insulin.
Conclusion: We have developed a simple protocol that produces effective human IPCs from human fat-MSCs *in vitro*. Our findings imply that functional IPCs generated by this method can be used in management of T1D in the future.
Keywords: Adipose Tissue, Mesanchymal Stem Cells, Diabetes, Differentiation

Ps-71: Pdx1 Overexpression Enhances Pancreatic Differentiation of Human Induced Pluripotent Stem Cells Derived from Fibroblast of Diabetic Patient

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Objective: Pancreatic and duodenal homeobox 1 (PDX1), a member of the homeodomain-containing transcription factor family, is a key transcription factor which is important for both pancreas development and mature β cell function. Induced overexpression of Pdx1 resulted in a significant upregulation of insulin and other pancreas-related genes. The generation of insulin-producing pancreatic β-cells from human iPS cells *in vitro* would provide an unprecedented cell source for cell transplantation therapy in diabetes without the ethical obstacle of embryonic stem cells and would bypass immune rejection.
Materials and Methods: PDX1 overexpressed hiPS cells were produced by Lentiviral transduction system and the infected cells were selected by Puromycin. A differentiation process was carried out according to kroon et al., 2008 protocol with some modifications that converts human induced pluripotent stem cells to endocrine cells that capable synthesizing the pancreatic hormones insulin, glucagon, somatostatin. This process mimics *in vivo* pancreatic organogenesis by directing

cells through stages resembling definitive endoderm, primitive gut-tube endoderm, posterior foregut, pancreatic endoderm and endocrine precursor which leads to cells that express endocrine hormones. We characterized the differentiation process in each stage at the RNA and protein levels using real-time PCR, western blotting, immunofluorescence and flow cytometry.
Results: The results indicated high expression level of each stage-specific markers including SOX17, FOXA2, and GSC in DE stage, HNF4A in PG stage, PDX1 and HNF6 in PF stage, NKX6-1, NGN3, NKX2-2, PTF1A in PE stage and pancreatic hormones such as insulin, glucagon, somatostatin were also detected.
Conclusion: These results demonstrated that overexpression of PDX1 is an important new strategy for the efficient generation of functionally immature insulin-producing β-islet cells from hiPS cells.
Keywords: Human-Induced Pluripotent Stem Cells (hiPSCs), Definitive Endoderm (DE), Primitive Gut-Tube (PG), Posterior Foregut (PF), Pancreatic Endoderm (PE)

Ps-72: The Role of Ascorbic Acid in Reprogramming of Breast Cancer Cells by miR-302/367 Cluster

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Objective: MiR-302/367 cluster is highly expressed in embryonic stem cells and its expression is reduced following differentiation. Several studies indicate that ectopic expression of this miRNA reprograms cancer cell lines to an embryonic stem cell-like state. These studies have shown that this process can suppress proliferation and induce apoptosis of cancer cells. Most of the current data indicate a positive effect for ascorbic acid on reprogramming of somatic cells. However there is no sufficient information on whether cancer cells are similarly affected, especially following miR-302/367 overexpression.
Materials and Methods: MDA-MB-231 and SK-BR-3 human breast cancer cell lines were cultured and transfected with miR-302/367 and mock vectors and then treated with ascorbic acid for 1-week and 4-week periods. Expression of reprogramming and pluripotency factors at mRNA level was evaluated by quantitative real-time PCR. Cell cycle analysis was performed by flow cytometry.
Results: In both cell lines, overexpression of miR-302/367 led to upregulation of pluripotency markers, especially at four weeks post-transfection. Ascorbic acid compromised the expression of some pluripotency markers in the cells which were transfected with miR-302/367 cluster. Flow cytometric analysis showed the inhibitory effect of miR-302/367 overexpression with or without ascorbic acid treatment, on cell cycle progression in both cells lines.
Conclusion: Results of this study indicate that ascorbic acid possibly acts with a different mechanism in cancer cells to somatic cells, in regards to reprogramming pathway of miR-302/367 cluster.
Keywords: Reprogramming, miRNA, Breast Cancer, Ascorbic Acid, miR-302/367

Ps-73: Effects of Mechanicaland Chemical Factors on Differentiation of Adipose Derived Mesenchymal Stem Cells into Smooth Muscle Cells

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Objective: In the past two decades, scientists have performed numerous researches on the application of mesenchymal stem cells in tissue engineering and regenerative medicine, the results of which indicate the high potential of stem cells in self renewal, proliferation and multi-linage differentiation, as well as being associated with low immunogenicity and responsive to their micro-environment. Effects of chemical environment on mesenchymal stem cell fate have been well documented. In recent years, it has been demonstrated that mechanical environment is of great importance in achieving functional cells for tissue engineering and cell therapy applications. In the current study, we evaluated the effects of both chemical and mechanical factors on stem cell differentiation into smooth muscle cells.
Materials and Methods: Rabbit adipose-derived mesenchymal stem cells were obtained from National Cell Bank of Iran. Experiments were carried out using three experimental groups, namely control (stem cells without any treatment), chemical stimulation and mechanical stimulation groups. In chemical stimulation group, cells were exposed to TGF-β_1 (with 10 ng/ml concentration in stem cell culture medium) for 24 h, while in mechanical stimulation group, a custom-made bioreactor was used to apply 5% cyclic uniaxial strain at 1Hz on stem cells for 24 h. The expression levels of α-SMA and SM22α as two specific smooth muscle markers were quantified using real-time PCR method.
Results: Real-time PCR results showed that cyclic uniaxial strain induced an almost 2-fold increase in α-SMA and SM22α expression, compared to the control group. TGF-β_1 treatment induced a 22-fold increase in α-SMA expression and resulted in a 35% enhancement in SM22α mRNA level in comparison with the control sample.
Conclusion: Gene expression analysis revealed that in addition to chemical factors, subjecting stem cells to uniaxial cyclic strain can induce the differentiation of stem cells into smooth muscle cells. Results can be applied in cardiovascular tissue engineering to obtain

functional smooth muscle cells.
Keywords: Mesenchymal Stem Cell, Cyclic Uniaxial Strain, TGF-β_1, Differentiation, Smooth Muscle Cell

Ps-74: The Effects of He/Ne Laser Irradiation on Rat Mesenchymal Stem Cell Characteristics and Apoptosis

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Objective: Low level laser irradiation is able to speed the healing process through increasing the cellular energy production and growth factor expressions which ultimately triggers cell proliferation and differentiation. This physical stimuli along with stem cell therapy can be an effective combination in the healing of injuries. The aim of this study is to optimize the effects of parameters of the 632.8 nm He-Ne laser irradiation on mesenchymal stem cells in order to achieve the best cellular proliferation and differentiation modulation as well as the least caspase activity.
Materials and Methods: Rat Bone Marrow Mesenchymal Stem cell (BMSCs) were isolated and cultured to the third-passage and divided into 11 groups at random. Each group were irradiated according to their specific irradiation protocol with various energy density ranging from 0.05 to 3 J/cm^2 and different intervals between exposures. Then, colony forming assay and population doubling time analysis were performed on irradiated MSCs. Treated MSCs were evaluated for apoptosis induction by measurement of caspase 3 activity. Besides, expression of markers such as CD44, CD90, CD45, and CD34 were compared before and after irradiation using immunocytochemistry method.
Results: The highest number of colonies (51.66 ± 3.82) and the least population time (5.30 days) were observed among the group that received o.1 J/cm^2 of laser radiation in four consecutive days which resulted in a total energy of 0.06 j. While there was no significant difference between markers expression before and after laser exposure, the aforementioned group showed the least caspase 3 activity.
Conclusion: Although a four-day low level exposure with the energy density of 0.1 J/c have two positive advantages including decrease of the apoptosis and enhancement of the proliferation of mesenchymal stem cells, it does not change in markers expression.
Keywords: Low Level Laser Therapy, Mesenchymal Stem Cells, Caspase 3 Activity

Ps-75: The Effect of Breast Cancer and Normal Adipose-Derived Stem Cells (ASCs) on The Proliferation and Apoptosis of Peripheral Blood Lymphocytes

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Objective: Mesenchymal stem cells are important for their presence in tumor microenvironment due to their immunomodulatory effects on both innate and adaptive immune responses. Herein, the immunosuppressive effects of adipose-derived stem cells (ASCs) from healthy donors and breast cancer (BC) patients on PBLs (peripheral blood lymphocytes) have been investigated.
Materials and Methods: Fragment of adipose tissue from normal and BC patients minced and digested with collagenase and stromal cells were purified by Ficoll gradient centrifugation. ASCs were prepared and characterized by flow cytometry. After co-culture of ASCs with PBLs, the proliferation and apoptosis of PBLs were determined by flow cytometry of CFSE stained cells and annexinV/7AAD dye labeling assay, respectively.
Results: Proliferation of PBLs co-cultured with normal and BC ASCs was approximately 2-folds lower than proliferation of these cells alone. Also, apoptosis of the PBLs co-cultured with both normal and BC ASCs was approximately 2-folds higher than PBLs alone. There was no significant difference between the effect of stage III and II BC ASCs on the proliferation of PBLs, while ASCs from stage II BC patients increase the apoptosis of PBLs 1.3-fold higher than ASCs from patients with pathological stage III.
Conclusion: It can be concluded that ASCs have the ability to induce immunosuppressive background in tumor microenvironment. Also different stages of breast cancer can change the effect of ASCs on the tumor infiltrative lymphocytes. ASCs may be introduced as appropriate therapeutic targets for breast cancer.
Keywords: Adipose-Derived Stem Cell, Peripheral Blood Lymphocyte, Proliferation, Apoptosis

Ps-76: Human Testis Derived Mesenchymal Stromal Cells Cryopreservation with The Potential for The Future Cell Therapy Applications

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Objective: Recent studies have shown that human testis derived cell cultures are neither pluripotent nor from germ cell origin whereas they are from mesenchymal stromal origin and multipotent. It would be desirable to set up a cryopreservation technique to preserve these cells for the future cell therapy applications in the case of people with testis cancer and male infertility. Here we have investigated the efficiency of conventional slow freezing cryopreservation method to preserve human testis derived mesenchymal stromal cells (hTMSCs).
Materials and Methods: A human testis from 90 years old man was obtained for research after fully consent ethical approval. The whole testis was minced to small pieces (1mm^3) and frozen in a 10% DMSO cryopreservation solution and transferred to -80ºC. The samples were transferred in the liquid nitrogen, the day after. After one month samples were thawed and cultured in two different culture conditions; DMEM/10%FBS and DMEM/20%FBS.
Results: 13 frozen human testis pieces were thawed and cultured. Initially small number of clusters and cells were observed, and after almost two weeks most of the flasks been confluent by hTMSCs. Human TMSCs have been passaged using trypsin/EDTA. From our experience 20% of FBS was more supportive for cell proliferation after cryopreservation.
Conclusion: Our data indicates that 10% DMSO cryopreservation solution would support hTMSCs preservation. Moreover, 20% FBS would be more supportive for the hTMSC cultures after cryopreservation thawing process. These data will lead itself for the potential of the hTMSCs in the future cell therapy applications. Further investigations are in progress for the identification of the hTMSCs before and after cryopreservation using different markers in a modified defined culture device.
Keywords: Stem Cells, Human Testis Derived Mesenchymal Stromal Cells, Cryopreservation

Ps-77: A549-Condition Medium Plus FGF2 Support Mouse Embryonic Stem Cells Differentiation into Alveolar Epithelial Type II Cells

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Objective: AETII cells are crucial to the natural regenerative process of the alveoli, and are considered as putative alveolar stem cells. Pluripotent stem cells (PSCs), like embryonic stem cells (ESCs), have potential to differentiate into cell types of all three germ layers, including lung epithelial cells. This study aims to differentiate mESCs into AETII cells with A549-condition medium (A549-C.M) plus FGF2 *in vitro*.
Materials and Methods: Royan 20 cells were induced to differentiate using adherent culture method and without the formation of embryoid body (EB). To induce differentiation, cells were converted in serum-free DMEM/F12 differentiation media. After endodermal formation by IDE2 on day 6, cells was treated with A549-C.M plus FGF2 for 9 days. a the end of day 15, the produced cells were analyzed by quantitative PCR, immunocytochemistry and flow-cytometry for markers of embryo, definitive endoderm (DE) and AETII cells. To confirm the generation of AETII-like cells on an ultrastructural level, TEM was performed.
Results: Differentiated cells displayed increased SP-A, SP-B, SP-C and SP-D (surfactant proteins, AETII cells specific markers) expression consistent with AETII cells production. We founded ~20.73% of ESCs displayed immunoreactivity to SP-C (unique feature of AETII cells which is commonly used to identify these cells from other lung parenchymal cells) after exposure to A549-C.M plus FGF2. There was minimal production of Foxa2 a marker of DE and Oct4, a marker of pluripotency. TEM exhibited ultrastructural features characteristic of AETII cells, including cytoplasmic lamellar bodies (LB) (organelles that contain pulmonary surfactant proteins). The presence of LB is a criterion traditionally used for the identification of ATII cells.
Conclusion: These results showed that efficiently generated AETII cells from mESCs can be used in future biomedical studies once safety issues have been overcome.
Keywords: Differentiation, mESCs, A549-C.M, FGF2, AETII Cells

Ps-78: Generation of Pulmonary Type II Cells from Mouse Embryonic Stem Cells Using A Novel Two-Step Differentiation Protocol

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Objective: The main functions of AETII-cells include the synthesis and secretion of surfactants, hyperplasia in reaction to alveolar epithelial injury and act as progenitor cells for AETI-cells, thus being able to renew the alveolar epithelium. We hypothesized that generation AETII-cells from mESCs *in vitro* could be achieved using a novel two-steps differentiation protocol.
Materials and Methods: The mESCs line, Royan-20, were maintained in the undifferentiated state in ''R2i''media. Then, cells were induced to differentiate without the formation of EB in SFD-media. For DE formation, IDE2 was added in the media for 6 days. For days 7–15 of the AETII-cells induction, the resultant DE was treated with new differentiation media containing, FGF2, hydrocortisone and A549-CM in SFD media for 9 days. At each stage (days 0, 6 and 15 of culture), the cells were collected and analyzed by qPCR, immunocytochemistry and flowcytometry for markers of embryo, DE and AETII-cells. To confirm the generation of AETII-cells on an ultrastructural level, TEM was performed.
Results: The mESC proliferated under the *in vitro* conditions and by days 6 and 15 displayed morphological characteristics of DE and AETII-cells, respectively. We found that Oct-4 was downregulated during differentiation and DE and AETII-specific genes were upregulated upon differentiation. On day-0, cells displayed increased Oct-4 expression but on day-6, differentiated cells displayed increased Foxa2 and Sox17 expression consistent with DE cells production. On day-15, differentiated cells displayed increased SP-A, SP-B, SP-C and SP-D expression consistent with AETII-cells production. We founded~36.13% of ESCs displayed immunoreactivity to SP-C (unique feature of AETII-cells) after exposure to FGF2, hydrocortisone and A549-CM. There was minimal production of Foxa2 and Oct-4. TEM analysis exhibited cytoplasmic-LB which is a criterion traditionally used for the identification of AETII-cells.
Conclusion: The current protocol is the first report demonstrating the AETII-cells differentiation by hydrocortison from mESCs-derived endoderm by IDE2. We have shown FGF2, hydrocortison and A549-CM to synergistically support the generation of AETII-cells from mESCs.
Keywords: mESCs, DE, AETII Cells, Differentiation

Ps-79: Injected versus Oral Cyclosporine for $CD3^+$, $CD4^+$, and $CD8^+$ T-Cells in Wistar Rats

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Objective: Cyclosporin-A (CsA) is a fungus-derived molecule that inhibits T-helper lymphocyte proliferation and has been largely used to downregulate cell-mediated immune responses during transplantation. CsA is widely employed clinically as immunosuppressive therapy in a variety of organ transplantations, as well as in the treatment of diseases of autoimmune origin. Moreover, CsA is frequently used in experimental transplantations in animal models. To test the hypothesis that may Low and high doses of CsA show any difference in lymphocyte ($CD3^+$, $CD4^+$, and $CD8^+$ T-cells) percentage , we used an experimental model for 10, 20 and 40 mg/kg doses of oral cyclosporine regimens in Rat.
Materials and Methods: Female rat of the outbred wistar stock, 6-8 weeks old, used in all of the experiments. Rats were randomized to two groups: 1. treatment and 2. control. The treatment group was administered 10 mg/kg per day dose CsA 1-3 weeks, 20 mg/kg 4-6 weeks and 40 mg/kg 6-9 weeks. Rats were weight and sacrificed weekly.
The peripheral blood of rat collected in EDTA-tube and RBC cells lysed with Lysis buffer. Cells stained with CD3-FITC/CD4-PE/CD8-Alexa Flour647 antibodies (ab Serotec Company) and incubated in 4°C at 30 min. Samples were detected with FACS AriaII (BD Bioscience) at 488 nm and 633 nm laser extention. Data analyses waa done with FACS diva software. All of the samples collect to 20.000 events.
Results: For statistical analyzes un-paired t-test was used to compare treated groups with their controls. At the end of the periods (3 weeks), there was no significant difference in T-cell count between different groups.
Conclusion: CsA is an immunosuppressive drug that has been widely used to inhibit immune responses during transplant rejection. Result of this study demonstrates that Flow cytometry was not able to detect effect of CsA on decreasing immune activity when administered at multi doses.
We recommend measure cytokine about immunosuppressive with ELISA test.
Keywords: Cyclosporin, T-Cell, Rat

Ps-80: Hepatogenic Differentiation of Human Breast Milk Derived Stem Cells, The Unique and New Source of Stem Cell

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Objective: In few recent years, such problems with well-known stem cell sources as low availability, pain-

ful access or limited proliferative ability have impelled scientists to take advantages of human breast milk stem cells (HBMSCs) in tissue engineering and regenerative medicine. However, only little information is available about hepatogenic differentiation potential of HBMSCs. In the present study, we examined the differentiating potential of HBMSCs into hepatocytes.

Materials and Methods: Human milk samples were collected in sterile tubes manually. HBMSCs were isolated by centrifugation and plastic adherence. After characterization, cultured cells exposed to hepatogenic media and differentiation ability was evaluated by biochemical and molecular experiments.

Results: The cultured HMSCs in presence of hepatic conditioned media significantly expressed mature hepatocyte mRNA such as albumin, alpha-fetoprotein, Cytokeratin-18, Cytokeratin-19, Cytochrome P2B6, Hepatic Nuclear Factor 4, Glucose-6-phosphatase and Cluadinin on day 30 of differentiation judged by RT-PCR. The production of ALB, CK-18, CK-19, and AFP proteins were assessed by fluorescent microscopy .The hepatocyte-like cells stained positively for CK-18, CK-19 and AFP at day 30. The glycogen granules were detected in the cytoplasm of cells exposed to hepatogenic media. Indocyanine green uptake and release was shown by differentiated hepatocyte-like cells.

Conclusion: Our study demonstrates that HMSCs as unique and novel population of stem cells. Therefore, human breast milk can be considered as a non-invasive of autologous source and has been suggested for treatment of liver diseases using hepatic tissue engineering.

Keywords: Human Breast Milk, Stem Cell, Hepatogenic Differentiation, Cell Therapy

Ps-81: Thymoquinone Enhances The Cytotoxicity of Temozolomide on U87MG Cell Line

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Objective: Glioblastoma multiforme is the most common and deadliest of malignant primary brain tumors in adults and average survival of patients is less than one year due to resistance to its chemotherapeutic agent (temozolomide). Recently there has been growing interest in naturally occurring compounds with anti-cancer potential. Thymoquinone, the main active constituent of black seed essential oil, has been used in traditional medicine for over 3,000 years. The main purpose of this study was to investigate whether thymoquinone is able to enhance the antiprolifertive effects of temozolomide on U87MG cell line.

Materials and Methods: U87MG cells were cultured in DMEM/F12 supplemented with 10% FBS without antibiotics at 37°C and 5% CO_2 in a humidified incubator. Colony formation assay was performed for evaluating the effects of temozolomide and thymoquinone alone and in combination on clonogenic capacity of the U87MG cells. Lactate dehydrogenase activity assay was performed for calculating the cytotoxicity of temozolomide and thymoquinone alone and in combination with the U87MG cells. Effect of temozolomide and thymoquinone on migration and invasion ability of glioblastoma cells were evaluated with scratch and transwell invasion assay. Effect of temozolomide and thymoquinone on DNA integrity of cells was detected using comet assay.

Results: Both temozolomide and thymoquinone, alone and in combination, decreased significantly colony formation ability of U87MG cells. Cytotoxicity value for combination of both temozolomide and thymoquinone is higher than each drug alone. Both temozolomide and thymoquinone, alone and in combination, decreased significantly migration and invasion potential of glioblastoma cells. Both temozolomide and thymoquinone caused double and single strand breaks in DNA and disrupted the integrity of DNA in U87MG cells.

Conclusion: Thymoquinone synergistically potentiated the efficacy of temozolomide against human glioblastoma cells.

Keywords: Glioblastoma Multiforme, Drug Resistance, Thymoquinone, Synergy

Ps-82: The Optimum Time for Neural Stem Cells Transplantation for Brain Stroke in Rats

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Objective: Brain stroke is one of the major reasons of disability these days around the world. There is no definite and effective treatment for recovering after brain stroke. Nowadays, cell therapy has been considered as an effective way to improve the neurological outcome after brain ischemia. Neural stem cells are a type of stem cells which are able to regenerate central nervous tissue. Here we have designed a study to distinguish the optimum time for neural stem cells transplantation after brain stroke.

Materials and Methods: The neural stem cells were isolated from 14 days- embryo rat ganglion eminence and they were cultured in NSA media (neurobasal media, 2% B27, 1% N2, bFGF 10 ng/ml, EGF 20ng/ml and 1% Pen/Strep). The isolated neural stem cells were injected intraventrically in different times (1 hour, 12 hours, 1 day, 3 days, 5 days and 7 days after stroke). The neurological outcomes were examined during 28

days after transplantation. Also, the ischemic areas were evaluated with H&E staining.
Results: The neurological outcome showed that the group which received neural stem cells 3 days after brain ischemia had the best neurological performance. Also the histological study presented that the group that received NSCs 3 days after stroke had the least amount of ischemic area.
Conclusion: Our result shows that the optimum time for neural stem cells transplantation after brain stroke is 3 days after that and it could provide better neurological out come and less brain damage.
Keywords: Neural Stem Cells, Brain Stroke, Different Time

Ps-83: The Protective Effects Mesenchymal Systemic Injection on Spinal Cord Injury in Rats

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Objective: Thoracolumbar spinal cord injury manifested by lower limb paralysis, bladder dysfunction and sympathetic nervous system disorders. These consequences could cause disability in the patients and societies. Unfortunately, there is no treatment for spinal cord injury. These days stem cells therapy has opened a new window for approaching to this health problem. In this study, we have examined the effects of pre- and post-spinal cord injury mesenchymal stem cells systemic injection on the rats' neurological outcomes.
Materials and Methods: The mesenchymal stem cells were obtained from rats' bone marrow by flashing the media and cultured in complete media containing DMEM, 10% FBS and 1% Pen/Strep. The cells were cultured for 1 week till they reached to 80% confluency. In 3rd passage the cells were harvested and labeled with PKH-26GL. The labeled cells were injected systemically via tail veins in two different groups (1 day before spinal cord injury and 1 day after spinal cord injury). The rats were followed for 3 months by neurological examination according BBB score.
Results: Both groups which received cell therapy recovered after 3 months in comparison with control group (P value<0.05). On the other hand, there was no significant difference between two groups that received cell therapy.
Conclusion: According to our results mesenchymal stem cells are capable to have protective effects on spinal cord injury rat model. MSCs injection before spinal cord injury could improve neurological outcomes as post injury MSCs could.
Keywords: Spinal Cord Injury, Mesenchymal Stem Cells, Pre Injury Injection, Post Injury Injection

Ps-84: Subcellular Proteomics of Human Embryonic Stem Cells

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Objective: Since the first human embryonic stem cell (hESC) line derivation in about 15 years ago, these eminent cells provided exquisite source for studding cell differentiation to human embryonic development. As long as one of the ultimate goals of hESCs applications is cell therapy, more detailed analysis is so critical and indispensable. Whereas the function of a protein is strongly associated with its localization in cell, a complete and accurate picture of the proteome of ESCs cannot be achieved without knowing the subcellular locations of proteins.
Materials and Methods: Freshly harvested cells have been fractionated into six subcellular fractions (cytoplasm, nucleus, mitochondria, plasma membrane, light and heavy microsomes).The efficiency of subcellular fractionation was validated by western blotting, PCA analysis, clustering followed by functional enrichment analysis and comparison with available subcellular localizations in GO and UniProt databases. All the fractions were subjected to gel-assisted digestion following by mass spectrometery analysis by Triple TOF Mass spectrometery and label free quantitation by IDEAL-Q. The function of three membrane proteins (ERBB4, ZDHHC13 and GGT1) in cell survival, cell cycle and pluripotency were also assessed by MTS, flow cytometery of BrdU labeled cells and qPCR, respectively.
Results: Subcellular fractionation of hESCs following by mass spectrometry analysis provided greater proteome coverage compared to whole cellular lysate and also a greater depth of signaling protein analysis. Cell cycle assessments for three membrane proteins shows that all of them played role in cell cycle regulation. Moreover, ERBB4 inhibition showed the impress of it on stemness maintenance.
Conclusion: Our report is the most comprehensive subcellular proteomic analysis in hESC which provides powerful data for further detailed studies.
Keywords: Subcellular Proteomics, Hesc, Membrane

Proteomics

Ps-85: Derivation of Epithelial-Like Cells from Menstrual Blood-Derived Stem Cells

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Objective: Human menstrual blood is easily accessible, renewable and inexpensive source of adult stem cells. Many studies indicate menstrual blood-derived stem cells (MenSCs) potential and feasibility in cell therapy in the case of some diseases. Differentiation ability of these cells into epithelial lineage especially compared to other stem cell types is not well understood. In this study, we evaluated the transdifferentiation capability of MenSCs into epithelial lineage cells in three-dimensional differentiating condition.
Materials and Methods: Menstrual blood samples were collected from 5 healthy female. After isolation and culture of MenSCs, in the presence of fibroin silk scaffold, differentiation into epithelial lineage was done. The parallel experiments were carried out to characterize epithelial cells specific markers such as Cytokeratin 14 (K14), Involucrin (IVL) and P63 using immunofluorescence staining and real-time PCR analysis.
Results: The differentiated stem cells showed the molecular characteristic of epithelial cells. Our real-time PCR analysis revealed the upregulation of specific markers including P63, K14, and IVL in differentiated MenSCs compared to undifferentiated cells. The expression of P63, K14 and IVL at protein level were shown by immunostaining method.
Conclusion: The results obtained in the present study showed the successful derivation of epithelial-like cells from MenSCs. The development of the method for efficient differentiation of MensSCs into epithelial lineage such as using scaffolds will enable us to get the fast and accessible source of epithelial cells to use in dermatological lesions.
Keywords: Menstrual Blood, Stem Cells, Epithelial Cells, Differentiation, Scaffold

Ps-86: Morphogenesis of Human Pluripotent Stem Cell Aggregates toward Pancreatic Progenitors in Suspension Culture

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Objective: Human pluripotent stem cells (hPSCs) are considered as a valuable cell source for modeling development of different cell types *in vitro*. Producing hPSCs-derived pancreatic progenitors (PPs) can help in studying human pancreas development and also modeling pancreatic diseases such as type 1 diabetes. In this study, the hPSC aggregates were induced into PPs during a stepwise protocol in the static suspension condition and the organoid-like structures were characterized for different pancreatic markers.
Materials and Methods: After producing hPSC aggregates in suspension culture condition, they were induced using a three steps protocol: cell aggregates received CHIR99021 and Activin A at the first step (for 3 days), FGF7 and LDN at the second step (for 6 days) and FGF7, retinoic acid, LDNand SANT at the last step (8 days). The resulted structures were characterized for gene and protein expressions by real-time RT-PCR and immunostaining, respectively.
Results: After 15 days of induction, differentiated aggrigates formed organoid-like structures. Immunostaining and flowcytometry analysis of these structures showed that more than 80% of differentiated cells expressed PDX1, the specific marker for pancreatic progenitors. Real-time RT-PCR results showed an upregulation of PDX1, PTF1A, NGN3 and INS genes in these cells.
Conclusion: Using these study conditions, we could produce organoid-like structures which expressed some of the PP marker genes and proteins. Further characterization of these structures is necessary to find the exact developmental stage of these cells and to investigate their capability for further differentiations *in vitro* and *in vivo*.
Keywords: Human Pluripotent Stem Cell, Pancreatic Progenitors, Differentiation, Organoid-like Structure

Ps-87: Histone Deacetylase Inhibitor Suppresses Neurosphere Formation and Induces Neural Differentiation of Human Fetus Neural Stem Cells

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Objective: Stem cells are a kind of cells which are capable of self renewing and differentiating to the other

cell types. Neural stem cells are a subtype of stem cells that could be isolated from adult and fetus central nervous system tissue and differentiated to three neural lineages containing neurons, oligodendrocytes and astrocytes. Histone deacetylases (HDAC) are some enzymes which modulate gene expression and cellular processes. The small molecule inhibitor of histone deacetylase is used in clinic for some disease such as cancer and they might have some neuroprotection effects due to previous studies.
Materials and Methods: The neural stem cells were isolated from ganglion eminence of 12 week-old human fetus and they were expanded in NSA media (DMEM/F12, 2% B27, 1% N2 and 1% Pen/Strep). the cells were passed after 1 week by using trypsin 0.05%. Cultured neural stem cells were differentiated to three neural lineages to confirm their potency. In passage 3, the HDAC inhibitor sodium butyrate was added to the culture media with concentration of 2 μM. After 1 week the neurosphere formation was assessed and compared to control group. Also the neural stem cells in different two groups were exposed and their viabilities were evaluated by trypan blue staining.
Results: Adding sodium butyrate as a Histone Deacetylase inhibitor could diminish neurosphere formation, also it could enhance neural differentiation of neural stem cells which was assessed by β-tubulin immunocytochemistry (P value is less than 0.05). Viability of the cells that contained sodium butyrate in their culture media was higher than control group after exposing.
Conclusion: Histone Deacetylase inhibitor could suppress the neurosphere formation; however it could improve neural differentiation and provide some resistance neurons to oxidative stresses.
Keywords: Neural Stem Cells, Histone Deacetylase Inhibitor, Neurosphere Formation, Neural Differentiation

Ps-88: Cytotoxic Effects of 3Mix Antibiotic on Human Primary Dental Pulp Mesenchymal Stem Cells

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Objective: The purpose of this study was to evaluate the cytotoxic effects of 3mix antibiotic on human primary dental pulp cells. A 3-antibiotic combination (3Mix) is widely used in endodontics for root canal disinfection, particularly in pulp revascularization procedures. whereas, use of 3Mix has been popular for a decade, the cytotoxicity of 3Mix has not been evaluated on human primary dental pulp cells. Therefore, the purpose of this study was to determine the cytotoxicity of 3Mix and each single antibiotic component of 3Mix on human primary dental pulp mesenchymal stem cells.
Materials and Methods: Human primary dental pulp cells were exposed to either 3Mix or to each single antibiotic component of 3Mix for 1, 3, 5, and 7 days. Cell viability was determined using MTT assay.
Results: 3mix concentration of 0.024 μg/ml in all experimental groups showed the lowest toxicity in dental pulp cells at 1, 3, 5, and 7 days .The less cytotoxicity was obtained after 0.39 μg/mL administration of 3Mix on day 7 Ciprofloxacin had higher cytotoxicity on the cells compared with other antibiotics.
Conclusion: 3Mix had cytotoxic effects on dental pulp stem cells in higher concentration. However, more studies are necessary to know mechanisms in cellular and molecular level.
Keywords: Cytotoxicity, 3-Antibiotic Complex, Primary Dental Pulp Cell

Ps-89: Comparison of Two Novel Sperm Selection Procedures: Zeta Potential versus MACS

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Objective: Density gradient centrifugation (DGC) technique and swim up method are mainly two sperm preparation procedures which routinely used in the Andrology labs for separation of sperm with normal morphology and motility. Sperm selection based on viability and normal morphology does not eliminate the chance for DNA damaged spermatozoa to be inseminated during ICSI (Intra cytoplasmic sperm injection). Therefore, novel sperm selection procedures have recently used for ICSI. In this study, we aimed to compare two novel sperm selection procedures: Zeta potential based on membrane surface charge and MACS (magnetic activated cell sorter) based on surface apoptotic marker in infertile men.
Materials and Methods: Semen samples were collected from 20 infertile men. Each sample was divided into three portions. One portion was washed with Ham's F10+10 % albumin (unprocessed sample), second portion was used to the "DGC- Zeta procedure" and the third portion was subjected for "MACS-DGC procedure". On each portion, percentage of sperm with abnormal morphology (papanicolaou staining), protamine deficiency (Chromomycin A3 staining) and DNA fragmentation (TUNEL staining) were evaluated and compared between portions.
Results: Percentage of sperm with abnormal morphol-

ogy, DNA fragmentation and protamine deficiency were significantly decreased in the MACS and Zeta procedures compared to unprocessed.
Conclusion: The result of this study suggested that using novel sperm selection procedures alone with DGC could be useful for treatment of infertile men with high percentage of DNA fragmentation and protamine deficiency.
Keywords: Zeta Method, MACS Method, Protamine Deficiency DNA Fragmentation, Morphology

Ps-90: The Role of over Expression of Kdm2b as An Epithelialization Factor in Transdifferentiation of Fibroblasts to Functional Hepatocytes-Like Cells

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Objective: Cell fate conversion of terminally differentiated cells by defined transcription factors, even between different lineages, is a new approach to produce new cells to repair or replace diseased and damaged tissues. Some reports have demonstrated the conversion of fibroblast cells to functional hepatocyte-like cells (HLCs), using forced expression of some hepatocytes specific transcription factors (TFs) like *Hnf4α* and *Foxa3*. Improving the efficacy of this kind of cell fate conversion by adding some extra TFs, using epigenetic modulators and growth factors, are very critical to get more functional hepatocyte. Epithelialization is important during hepatocyte development, so it seems that factors facilitate epithelialization may improve the process of fibroblast to HLCs conversion. To investigate the role of epithelialization factor during conversion of fibroblast to HLCs, we chose *Kdm2b* as an epigenetic modulator to facilitate mesenchymal epithelial transition (MET).
Materials and Methods: Here we used lentiviral vectors to over-express *Hnf4α* and *Foxa3* and *Kdm2b*, in mouse fibroblast cells. Transduced cells were grown in hepatic media with appropriate growth factors. Epithelial-like colonies formed after few days of transduction.
Results: Our result demonstrated that addition of *Kdm2b* to two reported factors could increase the numbers and areas of functional hepatocytes-like colonies. In addition, appearance of these colonies was faster than the colonies produced only with *Hnf4α* and *Foxa3* factors. On the other hand, hepatocyte-like colonies, without *Kdm2b* over-expression, were disappear too fast. Moreover, gene expression analysis showed that *Kdm2b* could increase the Hepatocyte specific genes such as *Albumin*, *E-cadherin* and *Cyp7A1*.
Conclusion: The results indicated that Kdm2b could play a considerable role to facilitate fibroblast to functional hepatocytes-like cells conversion.
Keywords: Direct Conversion, Hepatocyte-Like Cells (Hlcs), Epithelialization

Authors Index

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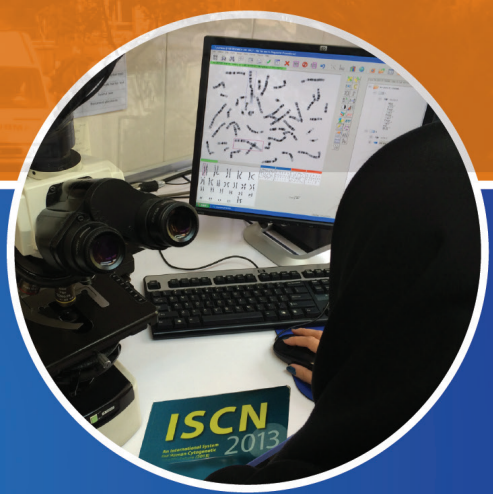

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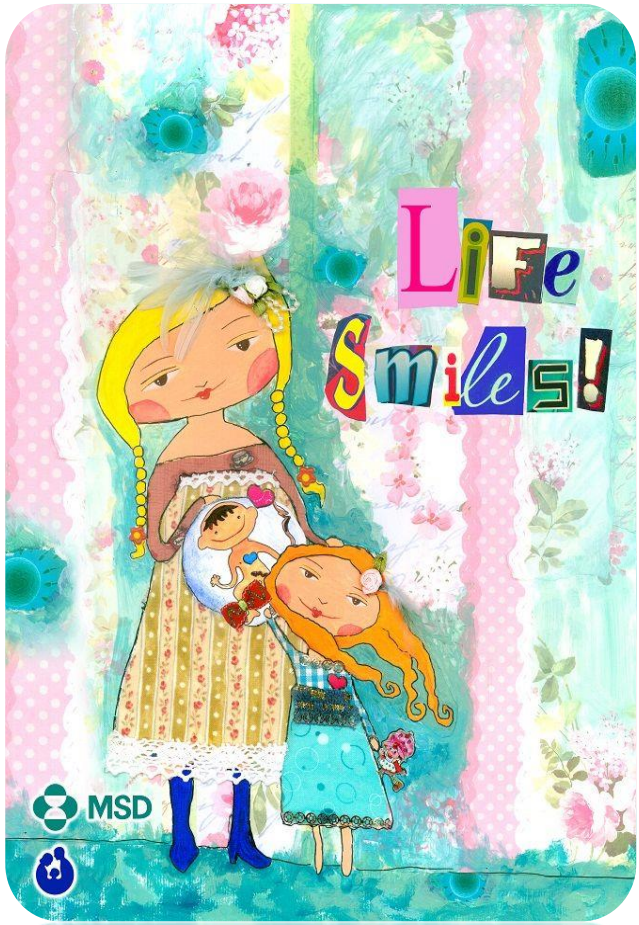

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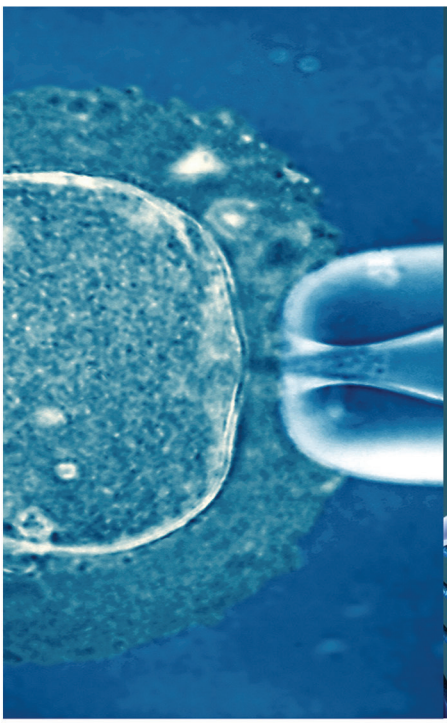

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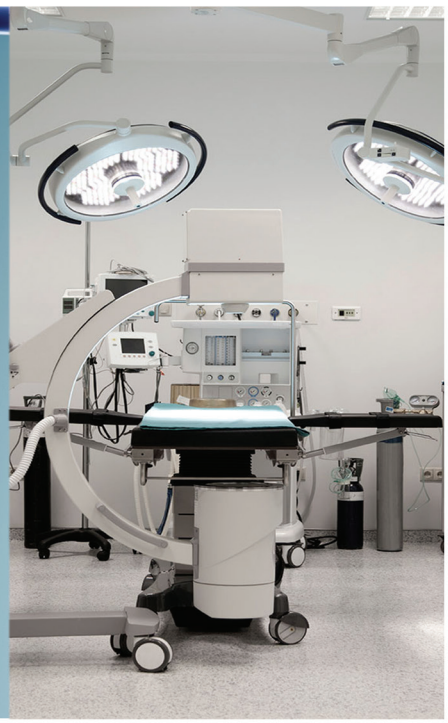

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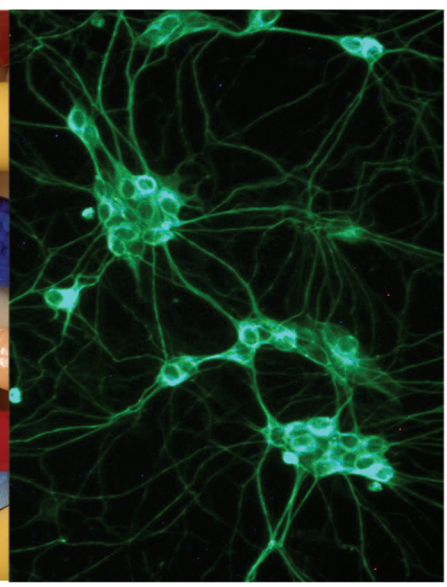

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شرکت ایران پنام

وارد کننده تجهیزات پزشکی و آزمایشگاهی

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Real Time PCR

3DEP Reader Cell Analysis Dielectrophoresis System

Multi chamber co2 Incubator

Rate Freezer for Stem Cell Cryopreservation

Micromanipulator IVF Lab Equipments

OSMOMETERS

CRYOGENIC VESSELS

Ultra-Low Temperature FREEZER

- **ESCO** سنگاپور، تولید کننده انواع هودها ی لامینار فلو-کلاس ۲ و ۳ ، CO2 انکوباتور فریزرهای ۸۶- وتجهیزات تخصصی IVF
- **PLANER** انگلستان، پیشرو در ساخت سیستم RATE FREEZER فریزرهاي قابل برنامه ريزي جهت IVF ، بانك اعضا و سلول
- **RI** انگلستان،معروفترین سازنده انواع سیستم هاي میکرواینجکشن و لیزرهچینگ
- **MEDLINE** انگلستان،تولیدکننده انواع شیکر , میکسر ،اسپکتروفوتومیتر و میکروسکوپ و...
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- **STATEBOURNE** انگلستان،سازنده انواع تانك هاي حمل و نگهداري ازت مايع
- **GONOTEC** آلمان، ارائه کننده سیستم هاي اسمومتر و کلرید متر
- **MEDITE** آلمان، سازنده پیشرفته ترین تجهیزات پاتولوژي، سیتولوژي و هیستولوژي
- **Grant** انگلستان، سازنده پیشرفته ترین تجهیزات آزمایشگاهی
- **Labplant** انگلستان،تولید کننده دستگاه هاي اسپري درایر (مولد پودر)
- **ASTELL** انگلستان، عرضه کننده انواع اتوکلاوها در حجم هاي مختلف
- **KUGEL** آلمان،تولید کننده سردخانه های بیمارستانی، میزهای کالبد شکافی و ابزارآلات کالبدشکافی
- **New Brunswick** انگلستان، تولید کننده فریزرهای ۸۶- درجه،شیکر، شیکر انکوباتور
- **Labtech** انگلستان ،تولید کننده نسل جدید دستگاه دی الکتروفورز جهت جداسازی سلول های بنیادی و سلول های سرطانی
- **PCR max** انگلستان ،تولیدکننده دستگاه های Real time PCR و گرادیانت PCR
- **Tech2art** دانمارک، تولید کننده انکوباتورهای رومیزی چند خانه و IVF Work station
- **JSresearch** کره جنوبی،تولید کننده دستگاه های اتوکلاو،آون،انکوباتور،انکوباتور سرد،شیکینگ انکوباتور،حمام آب گرم،اتاقک رشد گیاه
- **BIOBASE** چین،تولید کننده انواع هودهای آزمایشگاهی،،فریزرهای منفی ۸۶ درجه،فریزرهای منفی ۴۵ درجه یخچال های بانک خون، یخچال های آزمایشگاهی،سانتریفیوژ، اتوکلاو
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