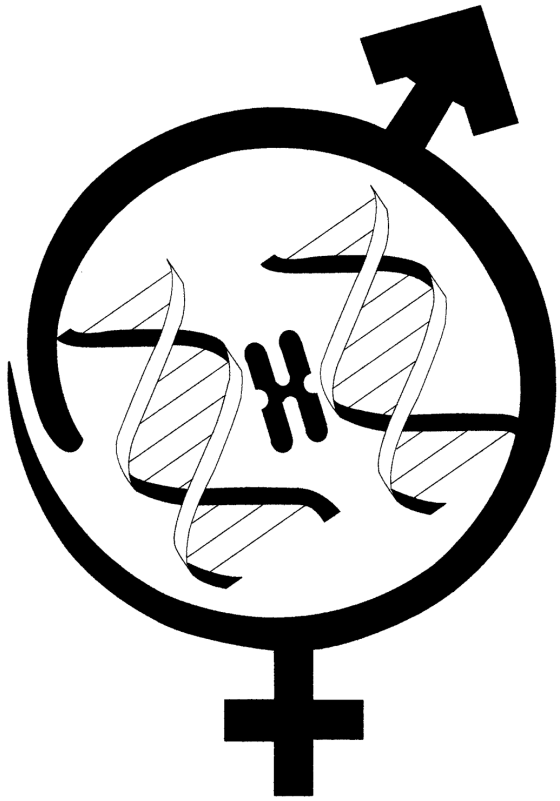
Abstracts of

Royan International Twin Congress

12th Congress on Stem Cell Biology and Technology

31 August - 2 September 2016



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).

1. Types of articles

The articles in the field of Cellular and Molecular Science can be considered for publications in ***Cell J***. These articles are as below:

A. **Original articles** are scientific reports of the original research studies. The article consists of English Abstract (structured), Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements, and References.

B. **Review articles** are the articles written by well experienced authors and those who have excellence in the related fields. The corresponding author of the review article must be one of the authors of at least three articles appearing in the references. The review article consists of English Abstract (unstructured), Introduction, Discussion, Conclusion, and References.

C. **Short communications** are the articles containing new findings. Submissions should be brief reports of ongoing researches. The Short Communication consists of English Abstract (unstructured), the body of manuscript (should not hold heading or subheading), Acknowledgements, and References.

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E. **Editorial** should be written by either the editor in chief or the editorial board.

F. **Images in biology** should focus around a single case with an interesting illustration such as a photograph, histological specimen or investigation. Color images are welcomed. The text should be brief and informative.

G. **Letter to editors** are comments made by our readers on recently published articles.

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Each article should be accompanied by a cover letter, signed and dated by corresponding author specifying the following statement: "The manuscript has been seen and approved by all authors and is not under active consideration for publication. It has neither been accepted for publication, nor published in another journal fully or partially (except in abstract form). I hereby assign the copyright of the enclosed manuscript to ***Cell J***. Corresponding author can also suggest three peer reviewers in the field of their article.

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1. Hardy-Weinberg Equilibrium (HWE) calculations must be carried out and reported along with the P-values if applicable [see Namipashaki et al. 2015 (Cell J, Vol 17, N 2, Pages: 187-192) for a discussion].
2. Linkage disequilibrium (LD) structure between SNPs (if multiple SNPs are reported) must be presented.
3. Appropriate multiple testing correction (if multiple independent SNPs are reported) must be included.

Submissions that fail to meet the above criteria will be rejected before being sent out for review.

Each of the following manuscript components should begin in the following sequence:

Title is providing the full title of the research (do not use abbreviations in title), full name(s), highest awarded academic degree(s), email(s), institutional affiliation(s) and email of all authors in English. Also you must send full postal address and mobile number of corresponding author.

Running title is providing a maximum of 7 words (no more than 50 characters).

Abstract must includ Objective, Materials and Methods, Results, and Conclusion.

Keywords, three to five, must be supplied by the authors at the foot of the abstract chosen from the Medical Subject Heading (MeSH). Therefore; they must be specific and relevant to the paper.

The following components should be identified after the abstract:

Introduction: This part includes the purpose and the rationale of the study. It should neither review the subject extensively, nor have data or conclusions of the study.

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Thesis:
* Name of author. Thesis title. Degree. City name. University. Publication date (year).
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Internet references
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2. Text of article and References prepared as stated in the "Guide for Authors" section.
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4. Cover Letter

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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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Congress Chairman

Massoud Vosough

Dear Colleagues

It is my great pleasure to invite you to the 12th International Congress on Stem Cell Biology and Technology, on 31 August to September 2, 2016, Tehran, Iran.

This event features three days of the best in stem cells research and regenerative medicine covering different aspects of basic and translational contents.

Since 2005, annual meetings on stem cell biology and technology have featured latest breakthroughs in this field and hosted eminent scientists from all over the world. National and international delegates presented their latest research projects and other participants found it as a novel opportunity to exchange ideas, discover, reacquaint with colleagues and broaden their knowledge. The scope and quality of the scientific exchange in previous meetings and increasing number of participants and received articles indicated and made this event as the premier stem cell research and instructional meeting in the region.

Royan institute was established in 1991 by the late Dr. Kazemi Ashtiani. Royan Institute for Stem Cell Biology and Technology (RI-SCBT) is one of the leading institutes conducting basic and translational research on stem cells, developmental biology and regenerative medicine.

In the upcoming congress (12th International Congress on Stem Cell Biology and Technology), the scientific programme consists of keynote speeches, plenary sessions, practical workshops and poster sessions.

In parallel to this event, 17th international congress on Reproductive Biomedicine will be held by Royan institute. On behalf of the Organizing Committee from Royan institutes, wish you a superb conference experience and a memorable stay in Tehran.

Massoud Vosough, M.D, Ph.D.
Congress Chairman of 12th Congress
on Stem Cell Biology and Technology

Is-1: Success Story: Royan Celltech Pharmed

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Is-2: A Novel Nanomaterial/Hydrogel Composite for Soft Tissue Regeneration

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Objective: Elastic hydrogels carry the potential to serve as scaffolds for various biomedical applications. Though some elastic hydrogels are biocompatible, most of the synthetic polymer-based elastic scaffolds lack bioactive sequences to promote cell adhesion or migration, which is important for their application in tissue engineering. Alternatively, recombinant proteinbasedpolymers such as elastin-like polypeptides (ELPs) are biocompatible and have been widely investigated for biomedical applications. Photocrosslinkable, protein-based biomaterials provide controllable and biocompatible crosslinking, permitting their use as tissue scaffolds and surgical materials.
Materials and Methods: Engineered ELPs in this study were designed by recombinant expression in *Escherichia coli (E. coli)*, followed by purification by inverse transition cycling. To fabricate the hydrogels, lyophilized ELPs were dissolved in phosphate-buffered saline (PBS) and photocrosslinked with UV light (360-480 nm wavelength, 850 mW) for 3 min. The swelling ratios of 10, 15 and 20% (w/v) ELP hydrogels were evaluated in PBS at 4°C and 37°C. Tensile and compressive cyclic tests were performed on ELP-based hydrogels using a mechanical tester (Instron model 5542) with a 10 N load cell. The *in vivo* biocompatibility of the ELP gel was investigated in a subcutaneous implantation model in rats. In addition, the use of nanoparticle-coated ELP hydrogel as a hemostatic material was evaluated in a rat liver bleeding model.
Results: Recombinantly expressed ELP hydrogels were shown to photocrosslink without additional modifications to the as-expressed protein sequence. The gels were extensible up to 420% strain and exhibited fatigue resistance in compression. The inclusion of thiol groups in expressed ELPs allowed for rapid photocrosslinking of hydrogels that maintained the elasticity and biocompatibility inherent in ELPs. The large extensibility of ELPs is important for the engineering of elastic tissues. The recombinant design of these ELPs allow for exceptional control over the presentation of bioactive peptide sequences, which can be used to improve cell viability, proliferation or promote specific cellular interactions *in vitro* or *in vivo*. *In vivo* examination revealed excellent biocompatibility, minimal degradation, and potential as hemostat scaffold when coated with silica particles.
Conclusion: The possibility for conjugation of bioactive sequences into recombinant proteins provides a wide range of tissue engineering applications that such a photocrosslinkable ELP could be tailored for, including cartilage regeneration, and vascular as well as ocular applications. Since crosslinking can be conducted in situ, the ELP can likewise function as tissue fillers, conforming to the shape of defects and subsequently being crosslinked stabilize the hydrogel.

Is-3: Engineering a Highly Elastic Surgical Sealant

Annabi N

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Objective: Approximately 114 million surgical and procedure-based wounds annually occur worldwide, including 36 million from surgeries in the US. Postoperative reconnection of tissues is crucial for restoring adequate function and structure. Sutures, wires, and staples are widely used for this purpose. Despite their common use in the clinic, these methods exhibit limitations when being applied in fragile and elastic tissues. To address these limitations, various types of surgical materials have been used for sealing, reconnecting tissues, or attaching devices to tissues. However, existing surgical sealant materials often feature limited adhesion strength, are toxic, lack appropriate mechanics, and do not function in wet environments1. Here, we developed a highly elastic protein-based sealant, which can be used to seal elastic tissues in the body after surgical procedures.
Materials and Methods: To fabricate the hydrogels, methacrylated recombinant tropoelastin (MeTro) was dissolved in distilled water and photocrosslinked with UV light2. The pore characteristics and swelling ratios of MeTro hydrogels with different MeTro concentrations and methacrylation degrees were evaluated. Tensile and compressive cyclic tests on MeTro hydrogels were performed using a mechanical tester (Instron model 5542). To study the adhesion strength of the MeTro gels to the native tissues, wound closure, lap shear and burst pressure tests were performed based on ASTM standards. The biocompatibility of MeTro was studied by *in vitro* tests and using an *in vivo* subcutaneous rat model. In addition, the functionality of MeTro as a lung sealant was tested *in vivo* using a rat lung model.
Results: We have developed highly elastic MeTro gels that can be photocross linked by using UV light for 10 sec. MeTro exhibited tunable mechanical properties depending on different MeTro concentrations and methacrylation degrees. Adhesion strength of MeTro sealant, measured by wound closure and lap shear tests showed properties superior to the clinical standard glues such as Evicel and Coseal. Improved cell viability and proliferation were achieved using MeTro gels *in vitro*. Subcutaneous implantation also showed excellent biocompatibility and material integration into the host environment. In addition, burst pressure of MeTro sealant covering a rat lung leakage was measured 7 days after surgery. The value of bust pressure for the MeTro-sealed lung was similar to that of healthy lung tissue, confirming that MeTro sealed the inci-

sion and promoted lung tissue healing.
Conclusion: A highly elastic, biocompatible, and biodegradable hydrogel-based sealant was engineered through photocrosslinking of a modified human protein. Our *in vitro* and *in vivo* data suggest that this material is superior to the existing products in the market and may generate a paradigm-shifting surgical sealant that, due to its excellent mechanical and adhesive properties, may not require additionally supporting sutures.

Is-4: Commercialization of ATMPs: Regulatory, Technical and Economical Challenges

Bos VD C

Director at Mares Ltd, Greven, Germany
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Objectives: Cell therapy concepts outside of hematopopietic transplantation/reconstitution have been put forward for the last 20 years and noticeable products include those for cartilage repair and burn treatments; recently, modified lymphocytes have gained prominence due to notable treatment successes.
Materials and Methods: In the European Community specific legislation is in force since 2008 covering cell- and gene therapy as well as tissue engineered products under the umbrella term Advanced Therapy Medicinal Products (ATMPs). In theory, this should provide a harmonized regulatory landscape for such products in Europe and accelerate the rate at which these are entering the market.
Results: In contrast to such expectations, only a handful of products have been approved via this route and, given the effort, size of the market and research funding provided to this sector, this is far from a success.
Conclusion: The question arises as to reasons as well as remedies and we will discuss contributing factors, namely technologies, efficacies, cost structures for allogeneic and autologous products as well as production strategies and will propose remedies.

Is-5: Stemming Vision Loss in Age Related Macular Degenerataion

Coffey P

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Is-6: Disease Modelling Using Induced Pluripotent Stem Cells

Coffey P

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Is-7: The CAR T Revolution in Hematologic Malignancies

Grupp S

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Chimeric antigen receptors (CARs) combine a binding fragment of an antibody with intracellular signaling domains. We have reported exciting data on CTL019 cell therapy expressing an anti-CD19 CAR. Infusion of these cells results in 100 to 100,000x *in vivo* proliferation, durable anti-tumor activity, and prolonged persistence in patients with B cell tumors, including sustained complete responses (CRs) in adults and children with acute lymphoblastic leukemia. This talk will update the audience on pediatric engineered cell therapy. Recent updates of our data in ALL show a 93% complete response rate and 79% overall survival at 1 year.
CTL019 cells can undergo robust *in vivo* expansion and can persist for over 4 years in patients with relapsed ALL, allowing for the possibility of long-term disease response without subsequent therapy such as transplant. This approach also has promise as a salvage therapy for patients who relapse after allogeneic stem cell transplant with a low risk of graft vs. host disease (GVHD). CTL019 therapy is associated with a significant CRS that responds rapidly to IL-6-targeted anti-cytokine treatment. This therapy has received Breakthrough Therapy designation from the FDA, and phase II multicenter trials are underway and have been completed.

Is-8: Utilization of Pioglitazone as A Novel Approach to Increase The Colony Formation Efficiency of Individualized Human Pluripotent Stem Cells

Ghaedi K[1, 2*], Kajabadi NS[1], Ghoochani A[1, 2], Peymani M[2], Kiani-Esfahani A[2], Hashemi MS[2], Nasr-Esfahani MH[2], Baharvand H[3]

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Objective: One problem in the development of human pluripotent stem cells (hPSCs) cultures is the vulnerability of these cells to undergo apoptosis or anoikis upon cellular detachment and dissociation. These cells undergo massive cell death, particularly after complete dissociation. The Rho-associated kinase (ROCK) inhibitor Y-27632 permits hPSCs survival upon dissociation. However, cloning efficiency is often still low. As, our previous studies showed that PPARgamma activation significantly enhanced the proliferation and survival rate of mouse embryonic stem cells, therefore, we hypothesized that the PPARgamma agonist, pioglitazone, might positively affect survival of dissociated single hPSCs and increase colony formation.
Material and Methods: We evaluated the effect of PPARgamma activation on cloning efficiency of single dissociated single hPSCs using pioglitazone. Flow cytometry analysis of cell cycle and apoptosis was performed on treated cells compare with the control. Gene expression analysis in dissociated single cells and colony of hPSCs was carried out. On the other hand Positive role of pioglitazone in colony formation was assessed by Western blotting and immunostaining

and co-immunopercipitation of memberanous beta-catenin. The relationship between Rho/ROCK signaling pathway and PPARgamma expression was also examined in a different cell type. Finally Pioglitazone and ROCK inhibitor Y-27632 maintenance of the pluripotency of hPSCs was examined by assessment of the respective markers in treated cells.

Results: Our data indicated that pioglitazone, a selective peroxisome proliferative-activated receptor-gamma agonist, along with Y-27632 synergistically diminished dissociation-induced apoptosis and increased cloning efficiency (2–3-fold versus Y-27632) without affecting pluripotency of hPSCs. Pioglitazone exerted its positive effect by inhibition of glycogen synthase kinase (GSK3) activity and enhancement of membranous beta-catenin and E-cadherin proteins. These effects were reversed by GW-9662, an irreversible peroxisome proliferative-activated receptor-gamma antagonist.

Conclusion: This novel setting provided a step toward hPSC manipulation and its biomedical applications.

Keyword: Human Embryonic Stem Cells, Colony Formation, Pioglitazone, Y-27632

Is-9: Lab Correlates of Highly Active Cell Therapy: Letting Our Patients Teach US in A Bedside to Bench Approach

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Relapsed/refractory leukemia, especially refractory disease, ALL in adults, and relapses after stem cell transplant, pose substantial challenges in both children and adults, with very little progress made in more than a decade. Targeted immunotherapy using chimeric antigen receptor (CAR)-modified T cells has emerged as a potent therapy with an innovative mechanism. Dramatic clinical responses, with our group observing complete remission (CR) rates as high as 90% (Maude et al NEJM 2014), have been reported using CAR-modified T cells directed against the B cell specific antigen CD19 in patients with highly refractory chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL). This lecture will seek to inform the audience about emerging topics in modified T cell therapies. Supraphysiologic T cell proliferation, a hallmark of active, T cell-engaging therapies, contributes to both efficacy and risk. The most notable toxicity is cytokine release syndrome (CRS), which poses a unique challenge for toxicity management. We will explore lab correlates of CRS and CRS prediction and treatment.

Starting with ALL, we will then discuss potential other application in pediatric diseases such as acute myeloid leukemia (AML) and neuroblastoma. AML may be targeted with CARs recognizing CD33 or CD123. CD123 is also a potential ALL antigen. Neuroblastoma may be targeted with the well-characterized antigen GD2, a disialoganglioside expressed on neuroblastoma cells. Preclinical data from ongoing studies will be presented showing the potential efficacy of alternative targets that may extend this therapy outside of B cell malignancy.

Is-10: Reconstitution of Insulin-Producing Cells by Islet Cell Type Interconversion

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By combining the targeted ablation of β-cells with the tracing of different lineages of islet cells, we have observed that massive loss of β-cells in adult mice results in the spontaneous engagement of a small fraction (<2%) of α- and δ-cells into insulin production. In juveniles, δ-cell reconstitution is strictly β-cell dependent, and extremely efficient. These unexpected naturally occurring α-to-β and δ-to-β cell fate conversions account for the b-cell regeneration and diabetes recovery observed in b-cell-depleted mice.

Several fundamental questions stem from these observations. For instance: Are the α- and β-cell populations heterogeneous regarding their plasticity potential? What signals trigger the conversion of α- and d-cells into insulin producers? Can islet cell plasticity be fostered and exploited therapeutically? Why δ-cells lose their tremendous plasticity potential in adulthood? Also, can human α- and δ-cells reprogram to produce insulin? An update of the studies on islet cell plasticity after injury will be presented.

Is-11: Success Story: Royan Biotech

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Is-12: Emerging Organ Models and Organ Printing for Regenerative Medicine

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Micro- and nanoscale technologies are emerging as powerful techniques for the development of highly organized and functional three-dimensional (3D) complex constructs. In addition, hydrogel biomaterials have been increasingly used in various tissue engineering applications since they provide cells with a hydrated 3D microenvironment that mimics the native extracellular matrix. In our lab we have developed various approaches to merge microfabrication techniques such as 3D bioprinting, photolithography, and microfuidic with hydrogel biomaterials for directing cell organization and generating complex 3D tissue. In this talk, I will introduce a number of bioprinting advances and highlight their potential for tissue engineering and biosensing applications.

Is-13: Microengineered Hydrogels for Stem Cell Bioengineering

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Engineered materials that integrate advances in polymer chemistry, nanotechnology, and biological sciences have the potential to create powerful medical therapies. Our group aims to engineer tissue regenerative therapies using water-containing polymer networks called hydrogels that can regulate cell behavior. Specifically, we have developed photocrosslinkable hybrid hydrogels that combine natural biomolecules with nanoparticles to regulate the chemical, biological, mechanical and electrical properties of gels. These functional scaffolds induce the differentiation of stem cells to desired cell types and direct the formation of vascularized heart or bone tissues. Since tissue function is highly dependent on architecture, we have also used microfabrication methods, such as microfluidics, photolithography, bioprinting, and molding, to regulate the architecture of these materials. We have employed these strategies to generate miniaturized tissues. To create tissue complexity, we have also developed directed assembly techniques to compile small tissue modules into larger constructs. It is anticipated that such approaches will lead to the development of next-generation regenerative therapeutics and biomedical devices.

Is-14: Generation of Induced Hepatocyte-Like Cells from Fibroblasts by Defined Factors

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Objective: The expandable patient–specific induce hepatocyte-like cells (iHLCs) that are generated through direct conversion can elucidate tumorigenic potential that induced pluripotent stem cells (hiPSCs) has. Thus, the generation of iHLCs has a potential to be used as a cell source for regenerative medicine and drug screening in liver disease.
Materials and Methods: To generated iHLCs, defined transcription factors were delivered to fibroblasts by lentivirus. The cell fate converting process was examined by observing the morphological changes. The expression of hepatic genes and proteins was examined by reverse transcription-polymerase chain reaction (RT-PCR) and immunofluorescence staining. To evaluate the functions of iHLCs *in vitro*, low-density lipoprotein (LDL) uptake assay, indocyanine green (ICG) test, periodic acid-schiff stain, and enzyme-linked immunosorbent assay (ELISA) were conducted.
Results: iHLCs were generated via direct conversion using define transcription factors in combination with lineage specific culture condition. iHLCs showed typical morphology of hepatocytes, hepatic markers and functions which include glycogen storage, LDL uptake, ICG metabolism, and Albumin secretion.
Conclusion: This study demonstrates a novel set of transcription factors in converting fibroblasts into functional hepatocytes which would be used in therapeutic applications.

Is-15: Direct Lineage Conversion of Fibroblasts into Oligodendrocyte Progenitor Cell for Spinal Cord Injury Application

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Objective: The generation of patient–specific oligodendrocyte progenitor cells (OPCs) holds great potential as an expandable cell source for cell replacement therapy as well as drug screening in spinal cord injury or demyelinating diseases.
Materials and Methods: We demonstrate that induced OPCs (iOPCs) can be directly derived from adult mouse fibroblasts by transduction with a single transcription factor, using anchorage-independent growth to ensure high purity.
Results: Homogenous iOPCs exhibit typical small-bipolar morphology, maintain their self-renewal capacity and OPC marker expression for more than 31 passages, exhibit high similarity in the global gene expression profile compared to wild-type OPCs, and give rise to mature oligodendrocytes and astrocytes *in vitro* and *in vivo*. Notably, transplanted iOPCs contribute to functional recovery in a spinal cord injury (SCI) model without tumor formation.
Conclusion: This study provides a simple strategy to generate functional self-renewing iOPCs and yields insights for the in-depth study of demyelination and regenerative medicine.

Is-16: Modeling Human Neural Tube Patterning with Microfluidic Gradients

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Knowledge about structural brain development is almost purely derived from studies performed in rodents or smaller model organisms, despite the fact that the human brain is much more complex and 2000 times larger than that of the mouse. This research bias is caused by the impossibility of performing dynamic studies on anatomical brain patterning in human embryos, resulting in a significant lack of knowledge on human-specific neural development.
Here, we build a simplified 3D model of the developing human neural tube *in vitro* using human embryonic stem cells (hESCs). Taking advantage of established knowledge on neural tube patterning, we have designed a closed microfluidic culturing chamber for differentiation of hESCs. In this chamber, the cells are exposed to a gradient of chemicals during the first days of differentiation to mimic the anatomical gradients of growth factors present in the embryo around the developing neural tube. We show that through differentiation of hESCs in the gradient chamber we are able to achieve progressive caudalization of neural identity, obtaining pure forebrain cells in the left side of the culture chamber to midbrain cells in the middle and hindbrain cells in the right side of the chamber, thereby mimicking the anatomical rostro-caudal organization

of the neural tube. Remarkably, we find the gene WNT1 to be very highly expressed in the area of the midbrain-hindbrain boundary in the culture chamber, indicating the formation of an *in vitro* equivalent to the anatomical structure of the Isthmic Organizer (IsO).
We apply this model to study region-specific effects of growth factors and key developmental genes, to show that the model can be used as a novel tool for studying anatomically relevant patterning of the early human brain. We envision that our model can be used to investigate how human brain development differs from that of other species in order to achieve an extraordinary degree of complexity.

Is-17: Bringing hESCs to the Clinic for Treatment of Parkinson's Disease

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Parkinson's disease (PD) is a neurodegenerative disorder involving loss of midbrain dopaminergic neurons in the Substantia Nigra. Recent developments have resulted in the generation of protocols for efficient patterning of human pluripotent stem cells into authentic midbrain dopaminergic cells which upon transplantation can restore motor symptoms in animal models of PD. In a joint European effort, we are now working to bring these hESC-derived dopaminergic cells to clinical trial for treatment of PD. A major challenge when developing such a therapy is that transplantation is performed with immature progenitors that undergo phenotypic and functional maturation after transplantation, and there is a lack of markers reliably predicting yield and maturation of the cells *in vivo*. This has resulted in high batch-to-batch variability in *in vivo* outcome. Therefore, we took an unbiased approach to identifying markers expressed in dopamine neuron progenitors that predict successful graft outcome in an animal model of Parkinson's disease through gene expression analysis of >30 batches of grafted hESC-derived progenitors. We found that commonly used markers did not accurately predict successful graft outcome, and identified a novel set of markers associated with the caudal midbrain, which correlated with high dopaminergic yield after transplantation *in vivo*. Using these markers, we developed a GMP differentiation protocol for highly efficient and reproducible production of transplantable dopamine progenitors from hESCs. With this new protocol, we will now be able to produce cell therapy for hundreds of PD patients from just a few flasks of differentiated cells.

Is-18: Modelling Human Brain Development in 3D Culture

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Is-19: Using Cerebral Organoids to Model Neurological Disorders

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Is-20: Adult Neurogenesis after Ischemic Stroke

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Stroke is caused by occlusion of a cerebral artery, which gives rise to focal ischemia with irreversible injury in a core region and partially reversible damage in the surrounding penumbra zone.
Stroke is the leading cause of disability in adult humans in developed countries. After ischemic stroke neurons are rapidly damaged and usually die but cellular loss can occur hours and days thereafter. Stroke increases stem cell proliferation in the subventricular zone (SVZ) and the generated neuroblasts migrate to the stroke-damaged area of the brain where they become mature neurons. This process may continue for several months and produce a significant number of new, functional neurons. The evidence of increased neuroblast production after stroke has also been demonstrated in human brain. A bulk of experimental evidence supports the idea that the stroke-damaged adult brain makes an attempt to repair itself by producing new neurons also in areas where neurogenesis does not normally occur, e.g., striatum and cerebral cortex. Knowledge about mechanisms regulating the different steps of neurogenesis after stroke is rapidly increasing but still incomplete. The functional consequences of stroke-induced neurogenesis and the level of integration of the new neurons into existing neural circuitries are poorly understood. In order to have a substantial impact on the recovery after stroke, this potential mechanism for self-repair needs to be markedly enhanced, primarily by increasing the survival and differentiation of the generated neuroblasts. Moreover, for efficient repair, optimization of neurogenesis most likely needs to be combined with promotion of other endogenous neuroregenerative responses, e.g., protection and sprouting of remaining mature neurons, and transplantation of stem cell-derived neurons and glia cells.

Is-21: Stem Cell Therapy for Stroke: Current Status and Future Perspectives

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Stroke is an acute neurodegenerative disorder and represents one of the leading causes of death and disability in adult humans in developed countries. After ischemic stroke neurons are rapidly damaged and usually die but cellular loss can occur hours and days thereafter. Stroke causes massive morbidity and mortality and tremendous economic and societal burden, being a leading cause of death and disability in adult humans.

In recent years, the recovery phase of stroke attracted much of the attention of researchers and clinicians, and currently is considered as most suitable target for the stroke therapy. This is justified by the long-term therapeutic window and also intrinsic plasticity-based mechanism of recovery which is operating in the brain and represents suitable target of the therapy. These therapeutic approaches would be initiated, many hours, if not days and weeks after stroke onset, with the intention of improving neurological function and not necessarily reducing the burden of the ischemic lesion. The aim of such therapeutic strategy is to enhance and accelerate the spontaneously operating self-repair/recovery mechanism. Stem cells have the capacity to generate neurons and glia cells which are lost in neurodegenerative diseases including stroke. Recently, stem cells of different origin have been tested for their ability to reconstruct the stroke-damaged forebrain and improve function after transplantation in animals models of ischemic stroke. The transplanted cells can survive and partly reverse some behavioral impairments. However, the underlying mechanisms of this improved recovery are unclear and there is little evidence for neuronal replacement. Besides cell replacement, stem cell-based approaches can also improve function by modulating inflammation, preventing neurons from dying, and increasing angiogenesis. These exciting laboratory findings should now be responsibly translated to the clinic. Some initial studies using cell therapy approaches have been performed in patients with stroke. There are several ongoing clinical trials in stroke patients, mainly using autologous bone-marrow derived cells. However, many issues remain before stem cell therapy can advance to full-scale clinical trials. These issues are (i) type of cells suitable for transplantation and their mechanisms of action, (ii) how to control proliferation, survival, migration, differentiation and integration of endogenous and grafted stem cells in stroke-damaged brain, and (iii) procedures for cell delivery, scaling-up, optimum functional recovery, and patient selection and assessment.

Is-22: Pin1-Catalyzed Signaling Regulation of Normal and Cancer Stem Cells

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Objective: Proline-directed Ser/Thr phosphorylation (pSer/Thr-Pro) is a central signaling mechanism in cell proliferation and transformation by inducing protein conformational changes. We have previously discovered that the conformation and function of many of these phosphorylated proteins are further regulated by a unique proline cis-trans isomerase Pin1. Such Pin1-catalyzed isomerization often acts as a novel molecular timer to help coordinate cellular processes synergistically towards one direction. Moreover, PIN1 is tightly regulated normally and its deregulation can have a major impact on the development and treatment of aging and age-related diseases notably cancer and Alzheimer's disease (Lu and Zhou, 2007, Nature Rev Mol Cell Biol, 8: 904). Our goal is to elucidate the role of Pin1-catalyzed signaling regulation in normal and cancer stem cells.
Materials and Methods: We analyzed Pin1 expression in normal and cancer stem cells in humans and in mice as well as developed conventional and tissue-specific Pin1 knockout mice and tissue-specific Pin1 overexpression mice to examine the role of Pin1 in the development of normal and cancer stem cells.
Results: We have discovered that Pin1 is expressed at the highest levels in normal and cancer stem cells, even in primary human cancer tissues. Pin1-/- mice develop normally, but display age-dependent depletion of normal stem cells in various organs, including testis, breast and brain, leading to widespread premature aging phenotypes, such as testicular atrophy, reduced fertility, failure of mammary glands to undergo massive proliferation during pregnancy and Alzheimer's disease. These Pin1 mutant mice are highly resistant to tumorigenesis even after overexpression of oncogenes such as Erb2 or Ras or deletion of tumor suppressors of such as p53. By contrast, Pin1 overexpression, which is prevalent in human cancers, promotes cancer and cancer stem cells by turning on and off at least 43 oncogenes and 21 tumor suppressors, respectively, many of which have a major role in cancer stem cells. In addition, Pin1 is shown to be required for maintaining protein stability of Nanog, Oct4 and c-Myc, three reprogramming factors critical for induced pluripotent stem cells, and for the reprogramming factors to convert human non-tumorigenic mammary epithelial cells into breast cancer stem cells. Importantly, Pin1 is a major drug target for the leukemia drug all-trans retinoic acid to treat acute promyelocytic leukemia by inhibiting leukemia stem cells, and for the antiprotozoal drug buparvaquone to treat Theileria-induced cell transformation in cattle.
Conclusion: Pin1 plays a fundamental role in regulating numerous signaling pathways in normal and cancer stem cells, and Pin1 inhibitors might have the desirable ability to simultaneously block multiple cancer-driving pathways in cancer and cancer stem cells.

Is-23: Biological Parameters Determining The Clinical Outcome of Autologous Cultures of Limbal Stem Cells

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Objectives: Limbal cultures restore the corneal epithelium in patients with ocular burns. We investigate biological parameters instrumental for their clinical success.
Materials and Methods: We report a long-term multicenter prospective study on 152 patients carrying corneal destruction due to severe ocular burns, treated with autologous limbal cells cultured on brin and clinical-grade 3T3-J2 feeder cells. Clinical results were statistically evaluated both by parametric and nonparametric methods.
Results: Clinical outcomes were scored as full success, partial success and failure in 66.05, 19.14 and 14.81% of eyes, respectively. The total number of clonogenic cells, colony size, growth rate and presence of conjunctival cells could not predict clinical results. Instead, the clinical data provided conclusive evidence that graft quality and likelihood of a successful outcome rely on an accurate evaluation of the number of stem cells detected before transplantation as holoclones expressing

high levels of the p63 transcription factor. No adverse effects related to the feeder layer have been observed and the regenerated epithelium was completely devoid of any 3T3-J2 contamination.
Conclusion: Cultures of limbal stem cells can be safely used to successfully treat massive destruction of the human cornea. We emphasize the importance of a discipline for de ning the suitability and the quality of cultured epithelial grafts, which are relevant to the future clinical use of any cultured cell type.

Is-24: Long-Term Follow-Up of *Ex Vivo* Gene Therapy of Junctional Epidermolysis Bullosa by Transduced Epidermal Stem Cells

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Objectives: Laminin beta3-deficient generalized Non Herlitz Junctional Epidermolyis Bullosa was the first genetic disease targeted by transplantation of autologous epidermal cultures originated from genetically modified epidermal stem cells. We demonstrate the feasibility, efficacy and safety of epidermal stem cell mediated *ex vivo* gene therapy.
Materials and Methods: The ongoing phase I/II clinical trial envisages the use of MLV-derived retroviral vector carrying the corrected *LAMB3* cDNA under the control of the viral LTR. Three patients were treated with autologous genetically modified epidermal cells cultured on plastic or fibrin and clinical-grade 3T3-J2 feeder cells. During post treatment follow-up several skin biopsies were taken from each patients to perform histological analysis, immunofluorescence, *in situ* hybridization and genome-wide analysis of the retroviral integration sites.
Results: The regenerated epidermis was apparently normal. The graft remained mechanically stable throughout the entire follow-up period and without blister formation, even upon shear force. Histological analysis showed a normal and fully differentiated epidermis with a normal dermal-epidermal junction. Electron Microscopy confirmed the presence of well-defined, organized hemidesmosomes comparable to those of healthy controls. We observed a proper expression and location of laminin 5 in the basal lamina. There was no evidence of dyskeratosis or abnormal proliferation and differentiation. In addition, the genome-wide analysis of the retroviral integration sites shows that the epidermal homeostasis of the corrected graft is sustained by few transduced stem cell. We obtained no evidence of clonal expansion or selection of specific integration events neither *in vitro* nor *in vivo*. *In situ* hybridization performed using vector-specific *LAMB3* probes showed homogenous expression of LAMB3 mRNA in all epidermal layers, confirming that the regenerated epidermis consists only of transgenic keratinocytes.
Conclusion: These data show that *ex vivo* gene therapy of Junctional Epidermolyis Bullosa is feasible and leads to full and permanent functional correction of the disease.

Is-25: Pancreatic Islet Cell Plasticity in Different Mouse Models of Diabetes

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We previously described that massive apoptosis of insulin-producing β-cells in mice leads to the spontaneous engagement of a small fraction of α- and δ-cells into insulin production. This α-to-β and δ-to-β-cell conversion accounts for the regeneration and recovery observed several months after diabetes induction with diphtheria toxin (DT) injection in transgenic mice bearing on β-cells the human receptor for DT. Islet cell plasticity was not previously observed in other experimental models of diabetes probably because of the lack of α- and δ-cell lineage tracing analyses. Two drugs, streptozotocin (STZ) and alloxan, are glucose analogues efficiently up-taken by β-cells, hepatocytes and kidney cells through the glucose transporter Glut2. Both compounds trigger diabetes as a result of β-cell necrosis: STZ is an alkylating agent inducing DNA breaks, and alloxan generates ROS free radicals. Here, we tracked the fate of α- and δ-cells in these two commonly used diabetes models. We first investigated the ability of the adult pancreas to regenerate β-cells from a- and δ-cells after partial β-cell loss by combining STZ or alloxan treatment with α- or δ-cell tagging. We found that 1 month after subtotal β-cell destruction, some α-cells started expressing β-cell-specific transcription factors, and a fraction of them also expressed insulin. δ-cells also reacted to partial β-cell loss by initiating insulin expression, but their response was detectable later, 1.5 months post β-cell destruction.
α- and δ-cell conversion were noticed thanks to the genetic labeling of transdifferentiated α- and δ-cells, half of which maintained glucagon and somatostatin expression. This could explain why α-to-β and δ-to-β cell conversion has been unappreciated in these models so far. In fact, when we investigated non-β-cell plasticity in non-transgenic C57BL6 mice, solely based upon hormone co-localization exploration, the number of converted cells was highly underestimated.
In summary, we show that insulin production in α- and δ-cells is independent from the mode of β-cell dismiss and occurs after either extreme or partial β-cell loss in different mouse diabetes settings.

Is-26: Bedside Practice, Hospital Exemption and Market Approval: Challenges for Cell Therapy Product Development

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Worldwide, the concept of Point-of-care (PoC) cell therapy is becoming increasingly attractive. A growing number of companies, medical practitioner and clinical facilities advertise PoC cell therapies for the treatment of various disorders, predominantly in the autologous setting. PoC cell therapy describes a continuous procedure for on-site patient care within one medical procedure, beginning from cell/tissue procurement, cell processing in or closed to the operation area, and ending with administering the resulting cell preparation.

Driven by the promise of clinical benefit, simplified manufacturing and reduced overall costs, PoC treatments could sustainable change the cell therapy landscape. Concomitantly, PoC therapies challenge the borderlines of clinical procedure vs medicinal product manufacturing, regulated pharmaceutical research, development and control vs medical practice, with extreme examples of unsound promises to patients. This presentation aims to provide an overview on the current regulatory setting for PoC cell therapy products with a focus on the situation in Germany and to facilitate the discussion on adequate supervision.
In situations of medical need and when no authorized product might be available the so-called hospital exemption clause can be applied to enable individual patients to receive "non-routine" ATMPs.
This regulatory concept will also be addressed.

Is-27: EU Regulation Framework for Licensing of Atmps

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This presentation aims to provide an overview on the development pipeline and regulatory approval process for Advanced Therapy Medicinal Products (ATMP) in EU and Germany, also addressing current challenges and upcoming modifications. Already in 2007 the European Parliament adopted the Regulation (EC) 1394/2007 on Advanced Therapy Medicinal Products, anticipating the strength of the research progress and increasing translational developments in stem cell-based and regenerative medicine, as well as other cell and gene therapy approaches. The Regulation classifies tissue engineered-products, somatic cell therapy- and gene therapy -products as pharmaceuticals and aims to provide a EU-wide framework for marketing of these products. Subsequent additional regulatory guidance and procedures have been developed in order to meet the specific scientific properties of ATMPs. On this legal basis, up to now six products out of several applications for ATMPs submitted to the European Agency (EMA) have been successful in obtaining a marketing authorization. To review the situation, the European commission (EC) has adopted a report on the experience since implementation of the ATMP-Regulation, re-opening the discussion on its Pro's and Con's.

Is-28: Translating New Discoveries in Beta Cell Development into Expandable Production of Beta Cells from Human Pluripotent Stem Cells

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Key challenges in stem cell-based therapy in diabetes include safe manufacturing of sufficient number of mature, functional beta cells *in vitro*. Furthermore, a better understanding the mode of action of growth factors/small molecules added to expand and differentiate heterogeneous human pluripotent stem cell (hPSC) cultures facilitates the conversion of experimental differentiation protocols into GMP conditions. Through systematic studies in mice and hPSCs we have addressed how key intermediate steps from the epiblast to the mature beta cell are regulated. Interestingly, many of the regulatory principles are conserved, which facilitated in depth mechanistic studies in the human system. By defining the mode of action of added growth factor/small molecule we have generated a robust protocol for coaxing hESCs into mature beta cells. To address the need for an expandable system we have identified unique cell surface markers in human multipotent pancreatic progenitors making it possible to purify and expand the progenitor population that normally is responsible for the growth of the pancreas. This system not only facilitates expansion of beta cell progenitors but it also removes undifferentiated hPSCs (and other cell types), which represents the major safety concerns in hPSC-based cell therapy. In addition, we have for the first time developed a system for re-seeding of purified beta cell progenitors and their efficient conversion into mature beta cells. Altogether, these results take us closer to the first clinical trials in diabetic patients using mature beta cells manufactured from purified expandable multipotent pancreatic progenitors.

Is-29: Autosomal Dominant Polycystic Kidney Disease: A Newly Treatable Disease

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Autosomal dominant polycystic kidney disease (ADPKD) is a familial multi-systemic disease that relentlessly progress to end stage renal disease in the majority of affected patients in their late adulthood. Approximately 7 million subjects are affected by ADPKD in Iran impacting health service. The disease is characterized by the slow but study growth of innumerous bilateral kidney cysts that replaces normal parenchyma. Progresses in our understanding of biological processes governing disease progression has led to the development of disease modifying treatment changing paradigm of ADPKD. Additional emerging therapies are currently tested that have the potential to stop the so far untreatable disease.

Is-30: Tuberous Sclerosis Complex: Elucidating Disease Mechanism to Design Treatment

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Tuberous sclerosis complex (TSC) is an inherited disease caused by mutations in two genes, hamartin and tuberin, that control growth and proliferation by suppression signaling through the mTOR complexes. The loss of function of these tumor suppressor molecules led to the growth of hamartomas in various organs, including brain, lung, kidney, gastrointestinal tract, eye and others. The elucidation of the biomolecular

mechanism of the mTOR complexes made it possible to develop a specific and efficient treatment.

Is-31: Cis-regulatory roles of lncRNAs in transcription regulation and stem cell differentiation

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Objective: Pervasive transcription in mammalian genome produces thousands of long noncoding RNA (lncRNA) transcripts whose functions are largely unknown. Identification and inference of functional lncRNAs are key challenges to understand the genome complexity and RNA-mediated gene regulation.
Material and Methods: We have rigorously investigated lncRNA functions during embryonic stem cell (ESC) differentiation by utilization of complementary genome editing, biochemial and single-cell approaches, including knock-out versus knock-in, deletion versus overexpression, knockdown versus cDNA rescue and CRISPR/Cas9 mediated RNA-tethering methods. We have discovered two important paradigms in lncRNA-mediated regulations of gene expression.
Results: We revealed a prevalent mode of cis-regulation of nearby transcription by divergent lncRNAs. Divergent lncRNAs transcribed oppositely from nearby protein-coding genes represent an interesting class of lncRNAs. They account for ~20% of lncRNAs in mammalian genomes, show strong correlation and coexpression with genes of essential regulatory functions in development, and have deeper evolutionary origin compared to intergenic lncRNAs. In-depth characterization of the divergent Evx1as/EVX1 locus revealed a direct role for the Evx1as lncRNA transcripts to promote EVX1 transcription in cis, and to regulate stem cell differentiation. At a single-cell level, early broad expression of Evx1as is followed by a rapid, high-level transcription of EVX1, supporting an upstream function of Evx1as. Mechanistically, Evx1as RNA binds to regulatory sites on chromatin, promotes an active chromatin state and interacts with Mediator. Remarkably, depletion of 75% divergent lncRNAs in various contexts, including pluripotency maintenance, lineage differentiation, reprogramming, human cancer and mouse zygotic development, led to downregulation of nearby genes. The effect of lncRNA-mediated cis-regulation may be more prominent but unlikely to be limited to the divergent lncRNA biotype. Previously we showed that the lncRNA Haunt binds to its own and downstream target HOXA genes on chromatin, and acts in cis to fine-tune HOXA induction during ESC differentiation. The Haunt DNA locus provides enhancers that are required for the activation of HOXA genes. Whereas Haunt RNA transcripts attenuate long-range chromatin interactions between the Haunt enhancers and the HOXA promoters, and serve as a "brake" to prevent aberrant activation of HOXA genes. The fine balance between the active and repressive functions of Haunt DNA and RNA, respectively, precisely controls the proper expression of the developmentally regulated HOXA locus, and contributes to orchestrated differentiation of ESCs.
Conclusion: We propose that lncRNA transcripts serve as a flexible cis-regulator to convey subtle regulatory information carried in the genome DNA. Cis transcriptional regulation by lncRNAs may be a general
theme of mamalian gene regulation. We further predict that lncRNAs, at least the subset of divergent lncRNAs, may participate in similar developmental or biological processes known to involve in nearby protein-coding genes through regulating their transcription. From this point of view, the functionality of thousands of uncharacterized lncRNA genes can be rapidly predicted on the basis of the knowledge of their neighboring genes. This functional inference may help to generate meaningful hypothesis to investigate lncRNA transcripts whose functions are largely unknown, and facilitate our overall understanding of non-coding portions of the genome.
Keyword: Divergent lncRNAs, ESCs, Transcription Regulation, Evx1as, Haunt

Is-32: Current Advances in Cell Encapsulation

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Objective: To give an overview of the advances in the past decade on cell encapsulation for treatment of endocrine disorders.
Materials and Methods: Transplantation of encapsulated and immunoisolated cells is proposed for treatment of endocrine disorders in which minute-to-minute regulation of metabolic agents is a requirement. Pharmaceutical intervention has often failed in this type of disorders.
Results: It has been established that producing biocompatible capsules for treatment of endocrine diseases requires a multidisciplinary approach. Physicochemical analysis of surfaces of hydrogel based capsules has demonstrated a large lab-to-lab variation in efficacy of producing biocompatible capsules. Major advances have been made in coupling polymers such as diblock or other complex systems to overcome lab-to-lab variations. Also major advances have been made in understanding the requirement that the intracapsular environment has to meet to facilitate survival of stem cell or other donor cell sources for long term efficacy of cellular encapsulated grafts.
Conclusion: Major advances has been made in encapsulation research during recent years. Combined physic-, chemical and biological approaches will facilitate the application of new generations of capsules for the treatment of disease. Stem cells play an essential role in this strategy.

Is-33: Towards Clinical Application of Encapsulated Islets for Treatment of Diabetes

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Objective: To give an overview of the application of encapsulated insulin producing cells for the treatment of Type I diabetes.

Materials and Methods: Exogenous insulin cannot prevent the development of diabetic complications nor can it prevent frequent hypoglycemia. This interferes with the quality of life of the patients and is also a major burden on the health care systems. A minute-to-minute regulating endocrine insulin source can prevent these complications and is therefore proposed as an ultimate solution for diabetics. However, transplantation requires the use of life-long immunosuppression which is associated with frequent infections. By encapsulating insulin producing cells in immunoisolating membranes rejection can be prevented and as a consequence immunosuppression is not required.
Results: Clinical application required a number of adaptations of the system. This is the consequence of novel insight into the human demands for mechanical and physical properties of the capsules as well as a documentation of the properties of the cell sources and applied polymers. Currently two types of devices have entered the clinical field; macro- and microcapsules. For macrocapsules an oxygen supply system is required as pancreatic islets have a high demand for oxygen. Both macro- and micro- capsules will be reviewed in view of clinical application.
Conclusion: The first experiences of human application of immunoisolating devices will be reviewed and discussed.

Is-34: Nanostructured Biomaterials for The Expansion and Differentiation of Stem Cells

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Nanostructure processing has been employed in creating synthetic cell culture substrates for the expansion and controlled differentiation of stem cells. These novel materials are biocompataible, non-immunogenic and pathogen-free since they are totally synthetic substrates free of proteins or products with animal origin. They are biochemically defined for reproducibility, and allow for the control of cell density and distribution. They have been employed for the successful expansion of stem cells, maintaining critical cell characteristics, such as pluripotency and viability over multiple passages.

Is-35: Nanostructured Biomaterials for Medical and Biological Applications

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Nanostructured materials have been developed for various medical and biological applications. They have been designed as stimuli-responsive drug delivery systems and sustained protein delivery systems. Nanocomposite systems have also been derived to provide simultaneous drug delivery and bioimaging functions as theranostic systems. Micellar nanocomplexes have been synthesized with green tea-based ingredients as unique carrier materials that offer synergistic therapeutic effects with the drugs to be delivered.
Nanotechnology has also been combined with microfabrication to obtain tissue engineering scaffolds and bioartificial assist devices. My laboratory has further developed nanosystems towards bioimaging, biosensing, diagnostics and drug screening applications.

Is-36: Stem Cell Therapy for Neurological Diseases

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Objective: To determine if the function of human stem cell-derived neurons, following transplantation into the brain, is regulated so that the therapeutic outcomes can be modulated accordingly.
Materials and Methods: Human embryonic stem cells were engineered by CRISPR to express the active or inhibitory from of DREADDs (designer receptor exclusively activated by designer drug), differentiated to midbrain dopamine neuron progenitors, and transplanted into the striatum of Parkinson's SCID mice.
Results: In culture, the membrane and action potentials of the DREADD-expressing midbrain dopamine (mDA) neurons were precisely regulated by a designer drug clozapine-N-oxide (CNO). Four months following transplantation into the striatum of Parkinson's disease mice, the human mDA neurons innervated the striatum extensively. Electrophysiological analysis indicated that the human mDA neurons form functional connections with and regulated host striatal GABA neurons via the D1 receptors. Behavioral analyses (rotation, cylinder, rotarod) indicated that the transplanted animals recovered from motor deficits. Importantly, the mouse motor recovery was reversed or enhanced after inhibiting or activating the graft function by CNO, respectively. Furthermore, activation of the grafted human mDA neurons dictated behavioral changes of the transplanted mice.
Conclusion: The function of human stem cell-derived neurons can be precisely regulated *in vitro* and following transplantation into the mouse brain. The ability to control the graft function enables refining the therapeutic outcomes remotely, thus raising the prospect of stem cell therapy.

Is-37: Human Stem Cell Models of Diseases

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Objective: To determine if human pluripotent stem cells, derived from patients or engineered to express disease related genes, can be used to model disease processes, identify underlying mechanisms, and develop platforms for drug discovery.
Materials and Methods: Induced pluripotent stem cells (iPSCs) derived from familial (D90A and A4V SOD1 mutations) amyotrophic lateral sclerosis (ALS) patients and embryonic stem cells (ESCs) that express the ALS mutations are differentiated to spinal motor neurons (MNs) as well as non-MNs

(control) and their pathological phenotypes are analyzed.
Results: Spinal MNs but rarely non-MNs exhibited neurofilament (NF) aggregation followed by neurite degeneration and MN death. These changes were associated with decreased stability of neurofilament-low molecular weight (*NEF-L*) mRNA and binding of its 3' UTR by mutant SOD1, and thus altered protein proportion of NF subunits. Such MN-selective changes were mimicked by expression of a single copy of the mutant *SOD1* in human ESCs and prevented by genetic correction of the *SOD1* mutation in patient's iPSCs. Importantly, conditional expression of *NEF-L* in the SOD1 iPSC-derived MNs corrected the NF subunit proportion, mitigating NF aggregation and neurite degeneration. Knockin of nanoluciferase or GFP into the *NEF-L* locus of ALS iPSCs by CRISPR faithfully reported the changes in NF in MNs, making them a useful platform for ALS drug discovery.
Conclusion: Neurofilament misregulation underlies mutant SOD1-mediated NF aggregation and axonal degeneration in ALS MNs. This finding suggests that human pluripotent stem cells, derived from patients or engineered to express disease related genes, can be used to model disease processes, identify underlying mechanisms, and develop platforms for drug discovery.

Is-38: Antibody against Early Disease Driver Cis P-Tau in Alzheimer's Disease and Brain Injury

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Objective: Alzheimer's disease (AD) and its related neurodegenerative disorder called chronic traumatic encephalopathy (CTE) share a common environmental risk factor, traumatic brain injury (TBI), and a common neuropathological hallmark, tauopathy made of phosphorylated tau (P-tau). However, little is known about how TBI leads to progressive neurodegeneration and how to stop this disease process at early stages. We have previously discovered a unique prolyl isomerase, Pin1 that protects against tauopathy in AD by catalyzing cis-trans isomerization of the phosphorylated Thr231-Pro motif in tau (P-tau). However, there was no tool available to visualize protein conformational changes and their biological function and pathological significance.
Materials and Methods: We have developed novel peptide chemistry that allows us to generate cis and trans-specific polyclonal and monoclonal antibodies against cis and trans P-tau. Using these antibodies, we studied the role of cis and trans P-tau conformation in the development and treatment of brain injury and Alzheimer's disease in cell and animal models as well as human diseased brains.
Results: We have shown that trans pT231-tau is the physiological form that promotes MT assembly, whereas the cis form is the previously unrecognized early pathogenic pretangle tau conformation that not only loses its normal function, but also gains toxic function, leading to tauopathy in AD. Moreover, we further discovered that cis P-tau is a precursor of tauopathy and early driver of neurodegeneration that directly links brain injury to Alzheimer's disease. Surprisingly, hours after TBI in mice and stress *in vitro*, neurons prominently produce cis p-tau, which causes and spreads cistauosis, including disrupting axonal microtubule network and mitochondrial transport, spreading to other neurons, and leading to apoptosis. Cistauosis eventually leads to widespread tauopathy and brain atrophy, characteristic of AD and CTE, but can be effectively blocked by cis antibody. Notably, treating TBI mice with cis antibody not only blocks early cistauosis, but also effectively prevents the later development of AD and CTE.
Conclusion: These results demonstrate that cis P-tau pathology, cistauosis, is a common early disease mechanism in TBI, AD and CTE, and that cis P-tau and its antibody may be useful for early diagnosis, prevention and therapy for these devastating diseases.

Os-1: Use of Crispr/CAS9 to Investigate the Function of Gene Mutations Found in Myeloid Malignancies

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Objective: Genome editing technologies have advanced significantly over the past few years to precisely manipulate the genome at specific locations. CRISPR/Cas9 has recently emerged as a powerful and versatile genome editing tool for genome engineering in various species. The adaptability, simpler assembly, higher specificity and efficiency of the CRISPR/Cas9 system make it now the most commonly used genome editing method.The myelodysplastic syndromes (MDS) represent a heterogeneous group of myeloid malignancies. The most common mutations found in MDS occur in genes that are epigenetic modifiers (e.g. ASXL1) or regulators of RNA splicing (e.g. SF3B1). Although it is clear that the common gene mutations impact both the pathophysiology and prognosis in MDS, we do not fully understand their role in MDS disease initiation and progression.
Materials and Methods: We used the CRISPR/Cas9 system to correct the ASXL1 homozygous nonsense mutation present in the KBM5 myeloid leukaemia cell line, lacking ASXL1 protein expression. We studied the impact of mutation correction on cellular function in Corrected cells.
Results: CRISPR/Cas9-mediated ASXL1 homozygous correction resulted in protein re-expression with restored normal function, including down-regulation of polycomb-repressive-complex-2 target genes. Significantly reduced cell growth and increased myeloid differentiation, providing new insights into the role of ASXL1 in human myeloid cell differentiation. Mice xenografted with mutation-corrected KBM5 cells showed significantly longer survival than uncorrected xenografts.
Conclusion: Given its successful application in the KBM5 cell line, the CRISPR/Cas9 system is used to correct recurrent gene mutations found in MDS haematopoietic stem and progenitor cells (HSPC). This study provides proof-of-concept for driver gene mutation correction via CRISPR/Cas9 technology in human cells and presents a strategy to illuminate the impact of oncogenic mutations on cellular function and survival. In addition, this technique provides a proof-of-concept for gene correction in primary adult HSCs derived from patients with a myeloid malignancy, paving the way for future gene therapy approaches.
Keywords: CRISPR/CAS9, Gene Mutations, Myeloid Malignancies

Os-2: An Old Remedy in A New Form: Egg White Macroporous Scaffolds

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Objective: Egg White (EW) is an excellent natural source of proteins like ovalbumin and lysozyme, with bioactive functionalities suitable for various biomedical applications.
Materials and Methods: Taking inspiration from ancient remedies, a simple strategy was developed to generate sponges from EW with low production cost and high yields. The developed processing technique is based on conversion of liquid EW solution into a gel, followed by freeze-drying of the gel into a solid-state scaffold with interconnected porous microstructure.
Results: EW scaffolds supported active metabolism, proliferation and migration of cells, thereby generating uniform cellular constructs. *In vivo*, subcutaneous implantation in mice revealed minimal immune reaction and efficient cell and tissue ingrowth. The scaffolds displayed an efficient engraftment and vascularization capacity, compared to standard collagen type I sponges used as reference material. These features were associated with significantly higher adsorption of the pro-angiogenic factor vascular endothelial growth factor (VEGF) and enhanced polarization of macrophages into a regenerative, M2-like phenotype.
Conclusion: This study validates macroporous EW sponges as novel scaffolds with growth factors binding potential for soft tissue engineering applications.
Keywords: Egg White, Macroporous Sponge, Crosslinking, Angiogenesis, Soft Tissues

Os-3: *In Vivo* Dmd Gene Editing in Muscles and Muscle Stem Cells of Dystrophic Mice

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Objective: Duchenne muscular dystrophy (DMD) is a X-linked genetic disorder that arises from frame-disrupting mutations in the DMD gene, encoding DYSTROPHIN. Lack of DYSTROPHIN expression destabilizes muscle fiber membranes, increases susceptibility to contraction-induced injury and drives muscle degeneration. Removing one or more exons from the mutated transcript can produce an in-frame mRNA and a truncated but still functional protein.
Materials and Methods: In this study, we developed and tested a direct gene editing strategy to recover DYSTROPHIN expression in the mdx mouse model of DMD by coupling clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 endonucleases delivered via adeno-associated virus (AAV) with paired guide RNAs flanking the mutated Dmd exon 23.

Results: We demonstrated precise excision of mutated exon 23 results in restoration of dystrophin reading frame and protein expression *in vivo* in both skeletal and cardiac muscles following local or systemic delivery. Dystrophin expression in AAV Dmd-CRISPR treated mdx mice was sufficient to partially recover functional deficiencies of dystrophic muscle. Finally, we demonstrated *in vivo* targeting of the mdx mutation in endogenous muscle stem cells, suggesting that AAV-CRISPR may provide a means to support ongoing repair of dystrophic fibers with corrected muscle precursors.
Conclusion: This study provides proof-of-concept evidence supporting the feasibility and efficacy of *in vivo* genome editing to correct frame-disrupting mutations in DMD.
Keywords: CRISPR, DMD, AAV

Ps-1: Camel Milk Casein Hydrolysates Promote Osteogenic Differentiation of Adipose-Derived Mesenchymal Stem Cells

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Objective: Bone diseases such as osteoporosis and fractures are among the main concerns for elderlies. In massive bone defects, bone grafting would be the best choice for bone healing; especially bone graft substitute materials with osteocunductive polymers and osteoinductive proteins. In this process the osteoinductive potential of the bone graft is perhaps the most important feature which stimulates mesenchymal stem cells to osteoblast differentiation. Nowadays there is a demand for finding novel osteoinductive agents which are more efficient and economic than common osteoinductive proteins such as BMPs. As a lot of studies have shown that milk and also milk proteins are beneficial for bone health, we hypothesized that milk protein hydrolysates may play a positive role in osteogenesis. Our previous studies proved that peptides derived from partial chymotryptic hydrolysis of camel milk casein have a significant impact on osteoblast differentiation.
Materials and Methods: In this study the hydrolysis was carried out at five different time intervals in order to find a product with the maximum effect on osteogenic differentiation of Adipose-derived mesenchymal stem cells. By unltrafiltration membranes, the hydrolysates were separated based on molecular weight and their osteoinductive capacity was measured by calcium content assay and Alizarin red-based assay.
Results: Our results showed that peptides with molecular weight of 5-10 KDa which were produced in 15 minutes hydrolysing, were significantly osteoinductive ($P<0.05$). The evaluation of the osteogenic markers expression such as RUNX-2, osteopontin and osteonectin by Real-Time PCR confirmed the previous results.
Conclusion: Our results suggest that these peptides act as osteoinductive agents which could be both neutraceutical and pharmaceutical preparations for bone regeneration.
Keywords: Osteoinductive Agent, Casein Hydrolysate, Adipose-derived Mesenchymal Stem Cell

Ps-2: *Ex Vivo* Mimicry of Bone Marrow Niche by 3D Co-Culture of Hematopoietic and Mesenchymal Stem Cells on DBM Scaffold in Microfluidic Chip

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Objective: The mold was fabricated with CNC technology then the bioreactor developed by soft lithography on PDMS. After that MSCs were isolated from bone marrow and characterized by immunological and multipotent differentiation assays, then seeded on DBM that was coated with collagen type I. Isolated HSPCs from cord blood using MACS method were co-cultured with MSCs for one week in cytokine free medium. After one week, cells were counted and analyzed by SEM, flow cytometry, colony forming assay and compared with 3D static culture and 2D conventional culture system.
Materials and Methods: The mold was fabricated with CNC technology then the bioreactor developed by soft lithography on PDMS. After that MSCs were isolated from bone marrow and characterized by immunological and multipotent differentiation assays, then seeded on DBM that was coated with collagen type I. Isolated HSPCs from cord blood using MACS method were co-cultured with MSCs for one week in cytokine free medium. After one week, cells were counted and analyzed by SEM, flow cytometry, colony forming assay and compared with 3D static culture and 2D conventional culture system.
Results: SEM analysis showed that MSCs efficiently attached to DBM/Col and covered it after 3 days of culture. Also HSC successfully attached in 7 days co-culture with MSCs. Co-cultured expansion of HSCs in static and dynamic culture was 2.06 and 5.78 fold respectively. Cell count increasing is not statistically significant in static culture but it was significant in dynamic culture system. Also flow cytometry indicates that CD34+ cells were markedly higher in dynamic culture than static. In colony assay analysis, maximum colony numbers belong to dynamic culture. By qRT-PCR assessment we observed that CXCR4 (Homing receptor for HSCs) was dramatically overexpressed in dynamic culture.
Conclusion: DBM is suitable material for mimicry of bone marrow microenvironment, it is the protein backbone of bone and highly porous that intensify surface/volume ratio. These results shows that when this scaffold utilizes with Collagen and MSC provide a suitable architecture and microenvironment for HSC expansion. Moreover, when this scaffold combines with dynamic microfluidic culture it can robust the results.
Keywords: Hematopoietic Stem Cells, Cord Blood, Expansion, Microfluidics, 3D Co-culture

Ps-3: Hepatocyte-Like versus Mesenchymal Stem Cells in CCl4-Induced Liver Fibrosis

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Objective: It is still a matter of debate as to whether *in vitro* mesenchymal stem cell (MSC)-derived hepatocytes may efficiently repopulate a host liver to provide adequate functional substitution. The aim of this study is to assess the efficacy and consistency of *in vitro* hepatic differentiation from Wharton jelly–derived MSCs, and to validate their therapeutic potential in experimentally induced liver fibrosis compared with nondifferentiated MSCs.

Materials and Methods: Forty adult male albino rats were divided into 4 main groups: (I) normal control group; (II) carbon tetrachloride (CCl4)-treated group (injected CCl4 solution twice a week for 8 wk); (III) MSC-treated group (a single intravenous dose of MSCs from human umbilical cord at the fourth week of induction of fibrosis); and (IV) hepatocyte-like stem cells (HLCs)-treated group (a single intravenous dose ofMSCs after *in vitro* conversion to hepatocyte at the fourth week of induction of fibrosis). Portal blood flow velocity and resistance, serum alanine transaminase, aspartate transaminase, albumin, and total bilirubin were measured. Liver homogenate was prepared for malondialdehyde, superoxide dismutase (SOD), nitric oxide (measured as nitrites), and TGFb (transforming growth factor beta) assessment. Assessment of human cells homing into liver rat and their function was performed using immunohistochemistry for detection of human hepatocytes and a-fetoprotein antigens.
Results: Significant elevation of serum alanine transaminase, aspartate transaminase, and bilirubin, liver malondialdehyde, nitrites, TGFb, fibrotic score, and significant reduction in serum albumin, liver SOD, and portal flow velocity in the CCl4-treated group were found when compared with normal rats. All these parameters significantly reversed in MSCtreated and hepatocyte-treated groups when compared with the CCl4-treated group. The MSC-treated group shows statistically better results in most liver function and oxidation parameters when compared with the HLC-treated group.
Conclusion: Human MSCs can differentiate *in vitro* into functional HLCs. Transplantation of both MSCs and HLCs was feasible and effective in a liver fibrosis rat model. However, MSCs were better in regaining liver function. Future studies should address strategies to improve long-term implantation of MSCs and HLCs in the host liver.
K*eywords:* Hepatocyte Like Stem Cells, Mesenchymal Stem Cells, Liver Fibrosis

Ps-4: The Mode of Action of Fucoidan on Breast Cancer Cell Line, and its Effects in Combination with Chemotherapeutic Agents

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Objective: Globally, breast cancer is a leading cause of death among women. Several studies proved fucoidan's anticancer potential, but few have measured its efficacy in combination with other anticancer drugs. Cisplatin, doxorubicin and taxol are first-line therapies in breast cancer. This study determined the cytotoxic, apoptotic and cell cycle distribution effects of fucoidan alone and in combination with cisplatin, doxorubicin and taxol in MCF-7 breast cancer cells.
Materials and Methods: The IC50 value of each drug for MCF-7 was measured with the MTT cytotoxicity assay. Apoptosis was determined with the Annexin VFITC/PI, active caspase 3-7-9, Hoechst 33342 and cell cycle assays. MCF-12A, a non-cancerous epithelial breast cell line was used as a control.
Results: Fucoidan significantly increased the cytotoxicity of the chemotherapeutic agents tested. Costimulation of MCF-7 cells with any of the chemotherapeutic agents and fucoidan increased apoptosis induction, caspase-3/-7 and caspase-9 activation, relative to untreated controls. Furthermore, fucoidan treatment resulted in G1 phase arrest of MCF-7 cells and accumulation of the sub-G1 population, as revealed by flow cytometry. Fucoidan strongly induced accumulation of MCF-7 cells in the G2/M and sub-G1 phases in various drug combinations. No significant differences for cytotoxicity and apoptosis or cell cycle profiles were found between fucoidan-treated and untreated MCF-12A cells.
Conclusion: Fucoidan is an effective antitumor agent, either alone or in combination with cisplatin, doxorubicin and taxol in MCF-7 breast cancer cells. These findings suggest that fucoidan is a candidate natural product for cancer combination therapies. *In vivo* cancer models are required to evaluate fucoidan's translational potential.
Keywords: Fucoidan, MCF-7, Apoptosis, Cytotoxicity, Flow Cytometry

Ps-5: MiR-184's Effect on Oligodendroglia Differentiation in Neural Human Progenitor Stem Cells

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Objective: In this study, we recruited the lentiviral based overexpression of miR-184 to enhance the differentiating process of human neural progenitor stem cells (hNPSCs) to oligodendrocytes.
Materials and Methods: we have examined our hypothesis by QRT-PCR, immunocytochemistry, luciferase assay and western blotting.
Results: QRT-PCR results showed the down regulation of target genes including Bcl2L1, LINGO1 and Sox1 and also up regulation of oligodendrocyte specific genes such as Olig2 and MBP. Western blotting and immunocytochemistry experiments results are also in accordance with QRT-PCR results.
Conclusion: The enhancer role of miR-184 in induction of NPSC's differentiation to oligodendrocytes have confirmed by our results. Also these results confirmed our both efficiency and temporal point of view. Currently, *in vivo* studies are under operation which hopefully confirm our *in vitro* study results.
Keywords: MicroRNA, Differentiation, Oligodendrocyte, Human Neural Progenitor Stem Cells

Ps-6: Embryonic Stem Cell-Specific miR-302/367 Cluster Modulates Transforming Growth Factor-β Signaling Pathway in Human Breast Cancer Cells

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Objective: MiR-302/367 cluster is highly expressed in human embryonic stem cells and it has been shown to suppress tumorigenicity of some cancer cells. It has also been shown to reprogram somatic cells through several pathways including modulation of transforming growth factor β (TGF-β) signaling pathway. TGF-β signaling is involved in cell growth, differentiation, apoptosis,motility and angiogenesis. It also promotes epithelial to mesenchymal transition (EMT) which favors tumor invasion. Here, we aimed to study the effect of miR-302/367 overexpression on several key regulators of TGF-β signaling in two invasive human breast cancer cell lines.
Materials and Methods: MDA-MB-231 and SKBR-3 cells were cultured and transfected with a lentiviral vector expressing miR-302/367 cluster and a mock construct encoding green fluorescent protein (GFP), using Lipofectamine 2000 and selected by puromycin. After RNA extraction and cDNA synthesis, the expression of TGFB1, TGFBRII, BUB1, RHOC, AKT1, MAPK1, MAPK14, SMAD2, SMAD3, was assessed by quantitative real time-PCR.
Results: There was significant increase in the expression of TGFB1 but TGFBRII, BUB1, RHOC, AKT1, MAPK1, MAPK14, SMAD2, SMAD3 were downregulated in MDA-MB-231 cells. In SK-BR-3 cells, TGFB1 and SMAD2 were upregulated but TGFBRII,BUB1, RHOC, AKT1, MAPK1, MAPK14 and SMAD3 were significantly downregulated.
Conclusion: Most of the TGF-β family members are significantly downregulated by miR-302/367 overexpression. This is in accordance with suppression of invasion by miR-302/367 cluster in the breast, skin and colon cancer cells which was previously observed by our group and supports the anti-tumorigenic function of miR-302/367 cluster in human cancer cells.
Keywords: miR-302/367, Breast Cancer, Reprogramming, Invasion, TGF-ß

Ps-7: Induced Pluripotent Stem Cell-Derived Podocyte Cells Transplantation Improves Mouse Model of Membranous Nephropathy

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Objective: Podocytes as part of the glomerular filtration barrier play a major role in the initiation and progression of both immune and non-immune mediated glomerular disease. Subepithelial immune deposition in glomerular disease causes nephrotic syndrome (NS) by podocyte disruption. Membranous nephropathy (MN) is an immune mediated and one of the most common causes of NS in adults leading to end stage renal disease. MN is thought to be caused by specific binding of Igs to podocyte-associated targets.
Materials and Methods: In this study, we efficiently differentiated mouse tail tip fibroblast derived iPSC into podocyte cells and characterized them. Then we stablished the mouse model of MN employing a generated purified rabbit anti-podocyte polyclonal antibody by intravenous injection 5 days after pre-immunization of female C57BL/6J mice. The urine, serum and kidney samples were collected for 60 days. Then, we transplanted the iPSC-derived podocytes into injured mice on day 10 in combination with mice kidney derived hydrogel.
Results: We showed that differentiation efficiency of iPSCs into podocytes increased when in the first step, iPSCs were differentiated into cells of the intermediate mesoderm following subsequent differentiation into podocytes. We have found that iPSC derived podocytes transplantation significantly decreases proteinuria to control level.
Conclusion: We herein suggest that transplanted iPSC-derived podocytes can improve CKD model-associated proteinuria and glomerular function by homing in glomeruli and replacing damaged podocytes.
Keywords: Membranous Nephropathy, Mouse Model, iPSC Derived Podocytes, Anti-podocyte Antibody, Proteinuria

Ps-8: The Effects of Hyperosmolarity on Chondrogenic Differentiation of Mesenchymal Stem Cells

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Objective: Mesenchymal stem cell (MSC) derived chondrocytes have been regarded as an alternative cell source for cartilage cell-based therapy as opposed to autologous chondrocytes derived from patients' non-weigh bearing cartilage. Medium osmolarity is of the factors affecting MSC differentiation pathways. Also, hyper-osmolarity is one of the key regulators of angiogenesis, calcification and inflammation. This study was conducted in order to investigate the effect of different osmolarity culture conditions on the phenotypic characteristics and proliferation pattern of MSC and also the possible pathologic effects of osmolarity on chondrogenesis.
Materials and Methods: MSC were isolated from adipose tissue. The isolated cells were then cultured under different hyper-osmotic conditions, using either NaCl as an ionic osmolyte, or sorbitol as an non ionic osmolyte and also an osmoprotectant or PEG as an organic osmolyte. The effect of hyper-osmolarity on MSC growth and proliferation was evaluated by MTT assay. Changes in gene expression levels for cartilage specific and pathologic markers (Collagen II, Versican, Sox9, CollagenX, MMPs) were monitored by RealTime-PCR. Alkaline phosphatase activity and calcium deposition were also determined. The potential of angiogen-

esis and inflammation was assessed by measuring VEGF and TNFα secretion in media using ELISA.
Results: MTT assay showed that in all of the culture conditions there was an increasing trend in the number of the cells with time. Comparison between control and hyperosmotic conditions showed higher expression of collagen type 2 in hyperpsmolar groups. RT-PCR results showed expression of Col II was up regulated in all hyperosmotic groups in comparison with Iso-osmotic condition. MMP9 gene was down regulated in all hyperosmotic conditions.
Conclusion: Hyperosmolarity enhances the expressions of chondrogenic markers, regardless of the type of osmolyte used.
Keywords: MSC, Stem Cells, Chondrogenesis, Osmolarity

Ps-9: Legal Challenges in Stem Cells Application: Codifying Legal Codes

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Objective: Stem Cells are indistinctive cells, which because of their capability to repair and self-treatment as well as the capacity of dividing into various types of cells, are regarded as an important issue of study. These cells are studied by scientists both in order to cure diseases and to research and study new diseases and treatments. This technology has being always challenged by a broad set of issues and discussions around the world. The treatment application of stem cells although is done after a long process of testing and studying, it has a short age and its results have not been discovered so far. In many cases, the treatment operations are only regarded as researches, for instance nobody knows what permanent effects a stem cell (especially from a strange one) affects on a human body. Also there are many factors about the beginning and prevalence of a disease and we cannot get a decisive conclusion only based on testing limited cases. In security, the first important thing is that the stem cell must be relief of any kind of cancer cells, virus, bacteria, etc. The second is that if stem cells remain in the same place they enter or move to other body tissues and make a new clone. The last but not least is that will these cells remain in the patient's body in 30 years without risk and dozens of questions must be observed in legal issues. It seems that the legal discussions related to the research and application of stem cells should be to the extent of scientific advances of the field and with logical balance in these two areas, we can ensure the health of society and not harm the patients.
Materials and Methods: Our method in the present article is to clarify questions and effects of treatment cells and express a set of strategies to develop binding rules, i.e. legal codes.
Results: Codifying legal codes would, in one hand, support the researches and professional centers and, in other, would protect the health and fundamental laws of human bodies and observe the moral, international and national guides.
Conclusion: Codifying legal codes would, in one hand, support the researches and professional centers and, in other, would protect the health and fundamental laws of human bodies and observe the moral, international and national guides.
Keywords: Stem Cells, Law, Codes, Treatment, Patient

Ps-10: Studing The Effect of Thymoquinone on Mouse Bone Marrow-Derived Mesenchymal Stem Cells Viability *In Vitro*

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Objective: Thymoquinone (TQ) is a volatile compound of some medicinal herbs. Despite extensive studies on the biological and pharmacological effects of TQ, but there is no report on its effects on stem cells, especially mesenchymal stem cells (MSCs). Given the importance of immunomodulatory properties of MSCs, as well as their applications in tissue regeneration, and also the pro-/anti-oxidant and immunomodulatory effects of TQ, it can propose that this compound may affect the behavior and function of MSCs. The present study was designed to investigate the effect of TQ on mouse bone marrow-derived MSCs viability *in vitro*.
Materials and Methods: MSCs were isolated from 4-8 week mice and were cultured in DMEM supplemented with FBS. The identity of the cells was confirmed by differentiation assays into osteoblasts and adipocytes. The effect of different concentrations of TQ (0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64μg/ml) on MSCs viability was determined by MTT assay at 24, 48, and 72h. Furthermore, morphological changes of the treated MSCs were evaluated under an inverted microscope.
Results: The results of this study showed that TQ induced a distinct dose- and time- dependent diminution of MSCs viability. The IC50 values of TQ on MSCs were determined as 8μg/ml after 24h, and 4μg/ml after 48 and 72h. Morphological observations also revealed that TQ reduced the number of MSCs in a dose- and time- dependent manner and prominent morphological changes were common after 24h of treatments with 4μg/ml of TQ, compared with untreated controls.
Conclusion: This study indicates that TQ had less toxicity on MSCs at low concentrations, thus it can propose that this compound may affect proliferation, migration and immunomodulatory properties of the cells. It may also induce pre-/anti-inflammatory phenotypes in MSCs, which can affect immune system and thus MSCs applications in regenerative medicine, however, different studies are required to support these effects.
Keywords: Thymoquinone, Mesenchymal Stem Cell, Viability, MTT Assay

Ps-11: Studying The Effect of Osmolarity on Chondrogenic Differentiation of Human-Mesenchymal Stem cells

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Objective: Human articular chondrocytes are osmolarity responsive and increase matrix synthesis under cartilage-specific physiological osmolarity.Despite its chondro-inductive effects, high osmolarity is one of the key regulators of angiogenesis and an inducer of calcium deposition and inflammation.the aim of this study was to monitor expression of chondrogenic, osteogenic and angiogenic markers, under three different hyperosmolarity(400,480,550 mOsm) conditions during chondrogenic differentiation of human adipose derived MSCs.
Materials and Methods: Expression of Collagen II, Sox9, Aggrecan,Runx2 and osteocalcin were determined using quantitative real time PCR. Production of ColII and X was investigated by immunocytochemistry. Glycosaminoglycan (GAG) production was also visualized by Alician blue staining. Production and secretion of VEGF and TNF-α was detected by ELISA.
Results: Hyperosmolairty enhanced the expression ($P<0.05$) of cartilage specific markers at gene expression level for ColII (400 mOSM) and Sox9 (480 mOSM). GAG production confirmed acquiring chondrogenic phenotype after *in vitro* differentiation. Pathologic symptoms as deduced from calcium deposition, alkaline phosphatase (ALP) activity, VEGF secretion and upregulation of osteogenic markers were observed under different osmolarity conditions.
Conclusion: Although applying hyperosmolarity to chondrogenic cultures can further improve chondrogenesis, it is not sufficient to inhibit angiogenesis and osteogenesis. Therefore, the current biochemical-physical differentiation protocol for chondrogenesis still needs to be optimized.
Keywords: Tissue Engineering, Cartilage, Angiogenesis, Mesenchymal Stem Cells

Ps-12: Induction of Apoptosis by MicroRNAs in Gliobalstoma Cancerous Cells with Mesenchymal Stem Cells Mediated Delivery

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Objective: Annually, 3.5 in 100,000 people are diagnosed with central nervous system (CNS) cancer, accounting for 1.9% of all new cancer cases and 2.3% of cancer deaths worldwide. Glioma, the most common neuroepithelial cancer, represents the majority of malignant brain tumors in humans. Among all glioma cases diagnosed, a majority belongs to grade IV, also known as glioblastoma (GBM).Glioblastoma multiform is a highly malignant brain tumor known for its invasiveness and aggressive resistance to standard treatment. Despite advances in surgery, radiotherapy, chemotherapy, and targeting therapy, the disease remains one of the most lethal malignancies in humans, and new approaches to improvement of the efficacy of anti-glioma treatments are urgently needed. A promising approach to cancer treatment is using microRNAs. MicroRNAs (miRNAs, miRs) are closely associated with biological processes of tumor cells as the key regulators by recognizing specific mRNA targets, and further mediating post-transcriptional inhibition of tumor related genes. Therefore, miRNAs may be used as optional therapeutic targets in tumors resulting from accumulation of multiple gene mutations and their interactions . In this study mesenchymal stem cells are used as a delivery vehicle, because mesenchymal stem cells have strong tropism and a potential to infiltrate the blood- brain barrier, they are the promising vehicles for gene delivery.
Materials and Methods: In the present study, we applied analytical and bioinformatics approaches to identify a set of microRNAs that its over expression correlates with apoptosis in glial tumors. We analyzed the impact of this microRNAs with bioinformatics tools such as GEO.
Results: Three miRs (miR-125a,miR181b,miR34a) which are down-regulated in glioblastoma cell lines that manual up-regulation of them exhibit significant apoptosis expression in glioblastoma were identified.
Conclusion: In next step glioblastoma cell lines (U-87 and U-251) are going to be transfected with microRNA-containing virus and subsequently apoptosis will be analyzed.
Keywords: MicroRNA, miR, Glioblastoma, Apoptosis, Mesenchymal Stem Cells

Ps-13: Small-Diameter Vascular Tissue Engineering: Studying The Biocompatibility of Amniotic Membrane Lysate (AML) Coated PLLA Scaffolds

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Objective: Despite advances in vascular surgery, vascular occlusion remains the leading cause of death worldwide. Although the gold standard for blood vessels replacement is autografts, the need for off the shelf blood vessel substitutes is undeniable. There are some main challenges to overcome regarding Tissue-Engineered Vascular-Grafts (TEVGs). Namely, the ability for long-term patency and their mimicry mechanical properties are issues yet to be resolved. There are two main approaches in vascular tissue-engineering: I) scaffold guided approach and II) cell-sheet-based techniques. Biodegradable polymers work as a temporary scaffold to support tissue growth. Scaffolds from the decellularized tissue skeletons to biopolymers and biodegradable synthetic polymers have been used for fabricating TEVGs. Human amniotic membrane (AM) is largely composed of collagen, laminin and fibronectin which are all sought proteins for best cell-scaffold interactions. The low immunogenicity and high healing power of AM makes it attractive for use in tissue engineering.
Materials and Methods: The present work is based on fabricating electrospun nanofibers using poly lactic acid and amniotic basement membrane Nano-composite scaffolds

were fabricated using Electrospun PLLA nanofibrous sheets following coating with AML. The surface of the scaffolds were plasma treated in order to increase the hydrophilicity of the composite sheet. Morphological and surface characteristics of the scaffold were investigated using SEM and FTIR. Mesenchymal stem cells were isolated from umbilical cord Wharton's jelly. MTT Cell Proliferation assay was also performed to assess the biocompatibility of the composite scaffold.
Results: The SEM images proved that the size of fibers ranged from 250 to 500 nm. We also proved the presence of amidic bands representing proteins in our scaffolds using FTIR. Comparison of cell growth on AML-coated PLLA scaffolds against untreated ones demonstrated an increase in proliferation rate of human mesenchymal stem cells on AM coated nanofibers.
Conclusion: The results showed that AML can be easily and safely applied to PLLA nanofibers surface in order to make bio-composite scaffolds with favorable properties for cell attachment and growth, which would potentially better support tissue remodeling and forming new vascular conduits.
Keywords: Vascular Tissue Engineering, Composite Scaffolds, Electrospinning, PLLA, Amniotic Membrane Lysate

Ps-14: What Is The Exact Detrimental Effect of Diabetes on Spermatogenesis; Renewal and / or Proliferation?

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Objective: Previous studies have shown that, diabetes adversely affects sperm parameters including, severe reduction in sperm count, motility, viability as well as DNA integrity. Various studies showed that, diabetes results in atrophied seminiferous tubules and diminishes the testicular endocrine status. Present study was done in order to uncover the exact mechanism(s) of action by analyzing the tubular renewal and differentiation indexes in testes of diabetic rats.
Materials and Methods: For this purpose, 24 mature male Wistar rats were divided in to; control (with no treatment) and diabetes-induced groups. The experimental diabetes was induced by streptozotocin (40 mg/kg). Following 20, 45 and 60 days after diabetes induction, the testicles were dissected out and were histologically analyzed. The seminiferous were analyzed for renewal and differentiation indices by examination of percentage of tubules with undifferentiated (with no spermatocyte type II and spermatid) and by analyzing the tubules with negative repopulation index (spermatogonia A relative to spermatogonia B). The results were presented in percentage and compared between groups.
Results: Observations demonstrated that, diabetes at early stages (20 days) significantly results in increased percentage of tubules with undifferentiated cell lineage. Meanwhile, long-time diabetes (45 and 60 days) stop-downs the renewal stages by remarkably ($P<0.05$) enhancing the percentage of tubules with negative repopulation index.
Conclusion: Our data showed that, diabetes affects the spermatogenesis by negatively affecting the differentiation pathway (at very early stages) and by adversely impacting the renewal system (at long-time types).
Keywords: Diabetes, Spermatogenesis, Renewal, Differentiation

Ps-15: Fetal Microchimerism in Mouse Caerulein-Induced Pancreatitis Model

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Objective: Microchimerism is the long-term persistence of allogenic cells population that transfer from the fetus to the mother, are called fetal microchimerism cells, and distribute in maternal tissues, where they show stemness activities and participate in tissue repair. The aim of this study was to evaluate the presence of fetal microchimerism cells in the exocrine part of caerulein-induced acute pancreatitis (AP) in mouse
Materials and Methods: AP model was obtained by intraperitoneal injection of 80 µg/kg of caerulein, 5 times with 1h intervals. Two days after delivery, the female wild type mice were mated with male EGFP+ and vaginal smears were checked for pregnancy. Sixty mice were divided into 3 groups: the virgin pancreatitis-induced animals, pregnant pancreatitis-induced animals mated with transgenic GFP mice, and pregnant sham animals. To prove pancreatitis induction, the blood samples were taken to assess amylase and lipase; and pancreases were removed from a subpopulation of each group for histopathological examinations after 6 h. The remaining mice were kept for 3 weeks. Their pancreases were used for histopathological exanimation, immunohistochemistry and PCR.
Results: EGFP+ cells were found in acini and around the blood vessels in the pancreas of pregnant pancreatitis-induced animals. They differentiated to adipocyte, acinar, and mesenchymal-like cells. PCR showed that 20% of the pregnant pancreatitis-induced animals contained EGFP gene. The histophatological study showed improvement in pancreatitis scores in the mice with pregnancy experience.
Conclusion: It seems that there is a correlation between the decrease in pancreatitis score and the presence of fetal microchimerism cells.
Keywords: Fetal Microchimerism Cells, Acute Pancreatitis, EGFP, Mouse

Ps-16: Myoblast Proliferation and Differentiation on Electroactive Polyurethane/siloxane Derived from Castor Oil for Cardiac Patch Application

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Objective: Our aim was to investigate the potential of electroactive polyurethane/siloxane derived from castor oil on proliferation of skeletal muscle C2C12 cell line and myotube formation without external electrical stimulation
Materials and Methods: A series of novel electroactive polyurethane/siloxane films containing aniline tetramer (AT) was prepared through sol-gel reaction of trimethoxysilane functional intermediate polyurethane prepolymers made from castor oil (CO) and poly(ethyleneglycol). Physicochemical, mechanical, and electrical conductivity of samples were evaluated and the recorded results were compared with that of non conductive sample. Also, the ability of the prepared samples on proliferation and differentiation of the C2C12 cell line was assessed.
Results: The optimized films were proved to be biodegradable and have tensile properties suitable for cardiac patch application. The embedded AT moieties in the backbone of the prepared samples preserved their electroactivity with the electrical conductivity in the range of 10-4 S/cm. The prepared films were also compatible with proliferation of C2C12 and had potential for enhancing myotube formation even without external electrical stimulation.
Conclusion: Proper selection of starting materials such as CO, a renewable raw material, and versatile polymerization strategy applied in this study helped to attain optimal physical,chemical, mechanical, and biological properties. Our data demonstrated that the inherently electrical conductive substrates are non-toxic and support cell proliferation ,attachment and differentiation . Our study highlighted the potential of embedding the electroactive moiety in the structure of the prepared samples to be used as cardiac patch. Results obtained in this study could extend the knowledge regarding the application of conductive polymers in the cardiovascular field.
Keywords: Polyurethane, C2C12 Myoblast, Cardiac Patch, Aniline Tetramer

Ps-17: Therapeutic Effects of Mesenchymal Stem Cell on Arterial Hypertension

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Objective: Systemic arterial hypertension (SAH), a polygenic and multi-factorial disorder, is a clinical syndrome characterized by persistent elevation of arterial pressure, is often associated with abnormalities such as microvascular rarefaction, defective, angiogenesis, and endothelial dysfunction.
Materials and Methods: Pubmed and google scholar search results (2004-2015), contains several articles and review articles in this field, showed that Mesenchymal stem cells (MSCs), which normally induce angiogenesis and improve endothelial function and exhibit the property of immune modulation, have been shown to affect and treat various diseases including SAH, pulmonary hypertension, endotoxin-induced hypertension during pregnancy or preeclampsia .
Results: In recent years, different studies showed therapy with mesenchymal stem cells injecting via the vena caudalis has been shown to cause partial or complete reversal of pathological characteristics of arterial pressure, especially pulmonary arterial hypertension (PAH). The therapeutic effects of stem or progenitor cell therapy, signiicantly induced a prolonged reduction (10 days) in arterial pressure, are considered to be (1) paracrine effects from stem or progenitor cells that had engrafted in the myocardium (or elsewhere), by compounds that have anti-inflammatory, antiapoptotic, and proangiogenic actions including reduced the levels of pro inflammatory TNF α and IL-1β, increased the levels of anti inflammatory IL 10, interleukin 1α (IL-1α), chemokine (C-C motif) ligand 5 (CCL5), and tissue inhibitor of metalloproteinase 1 (TIMP-1). Also (2) unloading effects on the ventricle due to stem or progenitor cell induced decrease in vascular resistance artery pressure by means of reducing in cardiac hypertrophy, an improvement in endothelium-dependent vasodilation response to acetylcholine and renin–angiotensin system, and an increase in skeletal muscle microvascular density compared to the vehicle and MSC groups. the transplanted cells were rarely found in the hearts and kidneys.
Conclusion: MSCs have a protective and therapeutic effect in an endotoxin-induced model of PE. This effect is likely elicited through the suppression of inflammatory factors, also potential therapeutic method for endotoxin-induced hypertension during pregnancy and adjusted renin–angiotensin system the specific mechanisms implicated in its action on blood pressure and electrolyte balance.
Keywords: Mesenchymal Stem Cell, Systemic Arterial Hypertension, Pulmonary Arterial Hypertension

Ps-18: Reconstruction of Calvarial Defects in Hypothyroid Rat Models Using Bio-Oss®-Collagen Gel Scaffold

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Objective: Thyroid hormones are essential for normal bone repair and in the absent of these hormones, delay will occur in bone mineralization and healing. Regarding to deficiency of thyroid hormone in hypothyroidism, osteoinductive carriers could be effective to maintain normal regeneration of skeletal defects. For this purpose, bioceramics (Bio-Oss®) together with type 1 collagen gel were used as a useful method for healing the critical-sized bone defects of hypothyroid rat models.
Materials and Methods: In present study, critical size defects (8 mm) were made in the calvaria of hypothyroid rats. Then the combination of Bio-Oss® and type 1 collagen gel

(Bio-Oss®-Gel) with the ratio of 0.02 mg/µl, as an osteoinductive scaffold, were used to filling these sites. Finally, the bone regeneration capacity of this carrier was investigated using multi-slice spiral computed tomography (MS-CT) imaging and histological analysis.
Results: 8 and 12 weeks after implantation, no mortality or sign of inflammation was observed in the site of defect. According to the results of MS-CT imaging analysis, a higher level of bone regeneration was observed in rats receiving Bio-Oss®-Gel compared to untreated group. Furthermore, histological evaluations were confirmed these findings. Impressive osseointegrartion was found in the samples were collected after 12 weeks post-transplantation.
Conclusion: In overall, it was demonstrated that combination of bioceramics and collagen gel enhanced bone reconstruction. Besides, 12 weeks after implantation can be the suitable time for filling the critical size calvarial defects in hypothyroid models. In conclusion, Bio-Oss® and collagen gel as a scaffold is an effective osteoinductive carrier to support bone mineralization deficiency due to hypothyroidism.
Keywords: Hypothyroidism, Critical-Size Bone Defect, Bioceramics, Bio-Oss®, Collagen Gel

Ps-19: Biocompatible and Biodegradable Super-Tough Elastomers Engineered by Supramolecular Interactions

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Objective: A perfect synthetic biomaterial should possess both tunable mechanical properties and appropriate tissue compatibility to be used as a biodegradable scaffold for tissue engineering. Through the biomaterials employed for engineering of vascular tissue applications, crosslinked elastomers are interesting. However, the majority of these elastomers are mechanically weak with a tensile strength of less than 2 MPa in wet state compared to the arteries and soft connective tissues.
Materials and Methods: By keeping in mind these limitations, we designed engineered linear alginate-based supramolecular ionic polyurethanes (ASPU) elastomer having physically reversible interactions in the solid state with superior mechanical and biological properties.
Results: ASPU as novel bioelastomers with high strength and toughness were developed under metal-free conditions. The Young's modulus and tensile strength of ASPU were tuned from 30 to 100 MPa, and 20 to 50 MPa, respectively. Furthermore, the ASPU exhibit a minimal creep deformation after 100 repetitive cycles. To the best of our knowledge, this is the first report that describes a linear PU can resist a large number of cyclic stresses without significant stretching. These bio-based elastomers engineered by ionic interactions are biocompatible and biodegradable. The biodegradation profiles of ASPU under *in vivo* conditions exhibited a similar trend in degradation profile of those compared to PCL, as an FDA-approved biomaterial with appropriate biodegradation. The proliferation rate of HUVECs on ASPU was higher compared to PCL and similar to tissue culture polystyrene (TCPS), as the gold standard, at both days 1 and 3. These biomaterials also demonstrate a rapid self-healing and recovery after rupture. In order to demonstrate the foreign-body reaction, we sought to investigate the *in vivo* cellular attraction and chronic inflammatory response.
Conclusion: The histological examination of subcutaneous transplanted ASPU in male Wistar rat after five months revealed low immunological response and low fibrosis.
Keywords: Biocompatible, Biodegradable, Elastomers, Supramolecular

Ps-20: *FOXO3A* and Signaling Pathway in The Rat Model of Parkinson's Disease

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Objective: Parkinson's disease (PD) is a neurodegenerative disorder characterized by protein aggregations in the cytoplasm of the dopaminergic neurons. Oxidative stress and mitochondrial dysfunction have a critical role in the pathogenesis of PD. Autophagy is a conservative mechanism in response to cell stress. Dysregulation of autophagy results in proteins aggregations and damage of organelles in neurodegenerative diseases. The aim of this study is evaluation of autophagy genes expression in PD.
Materials and Methods: Male wistar rats were divided into three groups as follows: control, sham (injection of ascorbate-saline solution into the left striatum) and lesion (injection of 6-OHDA dissolved in ascorbate-saline solution into the left striatum) groups. The apomorphine-induced rotation test was done one week before (base line) and four weeks after surgery. Then, rat's substantia nigra was extracted and RT-PCR was performed to detect expression of FoxO3a and the autophagy genes VPS34, Atg101, Atg14L, Atg13 and LC3.
Results: Assessment of apomorphine-induced rotation test indicated significant contralatelal rotations in the lesion group after four weeks compered to one week before surgery. By RT-PCR, lesion group expressed FOXO3A, VPS34, Atg14L, Atg13 and LC3 genes but did not express Atg101. Sham and control groups expressed all of these genes.
Conclusion: Expression of *FOXO3A* gene suggests that this gene has a role on induction of autophagy in PD through expression of LC3 and VPS34. Lack of Atg101 expression in parkinsonian rats may represents that the autophagy initiation process is probably deficient in PD.
Keywords: Parkinson's Disease, Autophagy, FoxO3a

Ps-21: P62 and Signaling Pathway in The Rat Model of Parkinson's Disease: The Role of Neural Stem Cells

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Objective: Parkinson's disease (PD), is primarily caused by the selective degeneration of dopaminergic neurons within the substantia nigra projecting to the striatum. Cell therapy is a therapeutic strategy to PD and based on the replacement of damaged neurons. Oxidative stress and mitochondrial dysfunction have a critical role in the pathogenesis of PD. Autophagy is a conservative mechanism in response to cell stress. Dysregulation of autophagy results in proteins aggregations and damage of organelles in neurodegenerative diseases. The aim of this study is evaluation of autophagy genes expression in PD.
Materials and Methods: Male wistar rats were divided into three groups as follows: control, sham (injection of ascorbate-saline solution into the left striatum) and lesion (injection of 6-OHDA dissolved in ascorbate-saline solution into the left striatum) groups. The apomorphine-induced rotation test was done one week before (base line) and four weeks after surgery. Then, rat's substantia nigra was extracted and RT-PCR was performed to detect expression of P62 and the autophagy genes Atg5،Atg12،Atg16L1،Atg10،GAPDH and LC3.
Results: Assessment of apomorphine-induced rotation test indicated significant contralatelal rotations in the lesion group after four weeks compered to one week before surgery. By RT-PCR, lesion group expressed P62،Atg5، Atg12، GAPDH and LC3 genes but did not express Atg10 and Atg16L1. Sham and control groups expressed all of these genes.
Conclusion: Expression of P62 suggests that this gene has a role on induction of autophagy in PD through expression of LC3. Lack of Atg10 expression in parkinsonian rats may represents that the autophagy initiation process is probably deficient in PD.
Keywords: Parkinson's Disease, Autophagy, P62

Ps-22: The Role of SHH Signaling Pathway in Dopaminergic Differentiation of Unrestricted Somatic Stem Cells (USSCs)

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Objective: Self-renewal and differentiation capacity of the stem cells have made them as potential tools for regeneration and replacement therapies in diseases.. Parkinson's disease (PD),a neurodegenerative disorder characterized by defect in dopaminergic neurons,has been candidate for the use of cell-based therapies . Due to the importance of signaling pathways in pathogenesis of PD, we sought to investigate the role of shh pathway in differentiation of Unrestricted Somatic Stem Cells(USSCs) towards dopaminergic neurons.
Materials and Methods: USSCs were therefore cultured in the medium containing neural differentiation factors (Retinoic acid 10 µM،IBMX 0.5 mM،Ascorbic acid 50 µM، bFGF 20 ng/ml) and SANT-1 1 µM (shh antagonist) /SAG 0.1 µM (shh agonist) for 3 days,and analyzed for the expression of CycD1(down stream target of shh pathway) and Nurr1(dopaminergic marker) by real time PCR.
Results: SAG increased the expression of CycD1 and Nurr1 significantly.The expression of both marker however was reduced significantly in the presence of SANT-1.
Conclusion: Altogether,we suggest that shh signaling pathway enhances dopaminergic differentiation
Keywords: shh Signaling, USSC, Dopaminergic Differentiation

Ps-23: Preparation and *In vitro* Evaluation of Poly (L-lactide) Electrospun Scaffolds Containing Magnetic Nanoparticles and Oyster shell for Bone Tissue Engineering

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Objective: Polymeric scaffolds containing deferent types of nanoparticles have recently attracted a significant attention in tissue engineering. In particular, increasing evidence shows that the synergistic effect of magnetic fields and magnetic responsive scaffolds can play unique roles in promoting bone repair and regeneration. In the present study, we fabricated nanofibrous scaffolds containing magnetic nanoparticles (MNP) and oyster shell (OS) and evaluated their potential for bone tissue engineering applications.
Materials and Methods: Nanofibrous scaffolds were prepared using the electrospinning of poly (L-lactide) (PLLA) solution incorporating MNP and HA. Furthermore, their mechanical and chemical properties were characterized and their capability to support proliferation and osteogenic differentiation of stem cells was evaluated *in vitro*.
Results: It was demonstrated that both MNP and OS were efficiently included in the fibers and efficiently distributed throughout the scaffolds. Incorporation of nanoparticles improved the hydrophilicity of the nanofibers. Mechanical properties of the nanofibers were significantly enhanced with the addition of both MNP and OS particles. The MTT assay also showed that the MNP-OS-PLLA had the ability to support the adhesion and proliferation of stem cells. Alkaline phosphatase activity and calcium content of stem cells demonstrated the enhanced osteogenic differentiation of stem cells on MNP-OS-PLLA scaffolds compared with control groups.
Conclusion: This study introduced the MNP-OS-PLLA scaffold as a type of graft substitute for bone treatment procedures. This scaffold was demonstrated to support stem cell proliferation and osteogenesis *in vitro* and holds promising potential for bone tissue engineering applications.
Keywords: Magnetic Nanoparticles, Bone Repair, Oyster Shell, PLLA, Stem Cell

Ps-24: Survey of Expression of Marker Genes in Sperm Maturation Include HAPRIN in Induced Human Hair

Follicle Stem Cells for Differentiation into Sperm Cells

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Objective: Azoospermia refers to cases that semen of men with no sperm or the count of sperm is zero. Due to low efficiency of previous treatments for this disease, today stem cell field are considered, as a new therapeutic approach for the treatment of male infertility. One of the best sources of stem cell is hair follicles that have stem cells including multipotent or pluripotent stem cells. Hair follicle stem cells (HFSCs) have high growth and proliferation capacity and can differentiate into various types of cells.

Materials and Methods: HFSCs after isolation from human hair follicles by using explant culture techniques were cultured in T25 flasks. The third passages were induced by sheep testicular extract conditioned medium in four experimental groups and HAPRIN expression investigated using western blotting technique.

Results: HFSCs after induction changed their shapes and construct sperm like head and tail and expressed HAPRIN proteins.

Conclusion: HAPRIN is believed to play a role in the acrosome reaction and fertilization, and in experimental groups has increasing trend that indicates; HFSCs could be differentiate into mature sperm like cells *in vitro*.

Keywords: Azoospermia, Stem Cells, Explant Culture, HAPRIN, Western Blotting

Ps-25: Systems Biology of Reprogramming: New Hopes to Improve Reprogramming Approaches, Regenerative Medicine and Cancer Therapy

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Objective: Cell identity is maintained through the complex interaction of transcriptional regulatory networks, chromatin regulators, non-coding and microRNAs, and signal transduction pathways. Direct reprogramming technology has revealed that a small number of core transcription factors are sufficient to establish a new identity in fully differentiated cell types and to control the gene expression programs. There is evidence indicating that direct cell fate conversions of somatic cells are not complete and the reprogramming products are not identical to their *in vivo* counterparts. Moreover, there is not a universal strategy for selection of fate-determining transcription factors.

Materials and Methods: Interestingly, several systems biology approaches (e.g. CellNet, KeyGenes and Mogrify) have been developed that can predict candidate factors by utilizing genome-scale technologies and multiple omics data.

Results: These approaches are capable of evaluating the exact identity of reprogramming products by comparing gene regulatory networks (GRNs) of the converted cells with their *in vivo* correlates. Moreover, these approaches can suggest methods to correct imperfect fate conversions and to improve reprogramming protocols for the generation of authentic functional cell types. For reprogramming purposes, computational methods predict and suggest a few transcription factors that can direct a specific transdifferentiation instead of examining a lot of transcription factors to identify the minimal influential set. Concerning the importance of cell fate conversion in biomedical research, further comprehensive analysis of the identity of the generated cell populations would improve the approaches toward the regenerative purposes, which safety and functionality of the cells are of essential importance. Suggestively, if these approaches are developed and cancer cells are included, they could be used in cancer therapeutics by suggesting strategies for the extinguishment of cancer GRNs.

Conclusion: Therefore, biological computational approaches offer new insight and strategies for improving reprogramming methods, tissue engineering/regenerative medicine and cancer therapy.

Keywords: Cellular Reprogramming, Gene Regulatory Networks, Biological Computational Models, Transdifferentiation, Systems Biology

Ps-26: Neuronal Differentiation of P19 Cells Induced by Royal Jelly in Conditioned Medium

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Objective: Degenerative diseases of the nervous system are caused by the nerve cells decadence. P19 carcinoma stem cells are capable of differentiating into cells of the three layers of the embryo. Compounds that induce differentiation of stem cells into the nerve cells, cause reduction in the disease symptoms. Royal jelly ability in neurogenesis leads to shape this hypothesis that this compound can enhance p19 cell differentiation into neuron cells.

Materials and Methods: Embryoid bodies derived from P19 cells were cultured with concentrations of 25, 50, 100, 150, 200 and 300 mg / ml royal jelly treatment. Differentiated cells stained with Cresyl violet were counted with graticule grid. Immunoflourescence showed the expression of specific markers for neuronal cells in differentiated cells.

Results: Of the cell was correlated with increased level of royal jelly concentreation ($p > 0.005$).The highest level of differentiation was observed.in concentration of 200 mg /ml of royal jelly. Immunoflourescence showed expression of specific markers of neuronal cells in differentiated cells.

Conclusion: The results of this study show that P19 cells are capable of differentiating into neural cells and might be used as a source for cell therapy of neurological disease. Furthermore, royal jelly might alternatively be used as a compound in treatment of degenerative nerve diseases.

Keywords: Carcinoma Stem Cell, Differentiation of Neural Stem Cells, Neural Marker

Ps-27: Beta Cells Derived From P19 Embryonal Carcinoma Stem Cells, in Response to Different Concentrations

Of Glucose Function Assessment

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Objective: It has been proposed that approximately 150 million people worldwide have diabetes mellitus, and 5-10% of those suffer from type 1 diabetes, for which injections of insulin are unavoidable. Transplantation of pancreatic islets for type 1 diabetes is a promising therapeutic strategy however, an inadequate supply of donor islets is a major obstacle. Thus, stem cells are being considered as a potential source for generating insulin-producing cells.P19 embryonal carcinoma (EC) are developmentally pluripotent cells. These cells can differentiate into all cell types under the appropriate conditions.
Materials and Methods: The cells were grown using low-attachment dishes to induce embryoid body (EB) formation. The resulted EBs cultured in a medium containing 3% fetal bovine serum (FBS), supplemented by concentration of 50, 100, 200 and 300 µg/mL mouse pancreas extract (MPE). Dithizone (DTZ), a zincchelating agent known to selectively stain pancreatic beta cells, was used to detect insulin-producing cells. In addition, insulin production and its secretion was examined using an ELISA.
Results: We recently reported the appearance of the islet-like cellular clusters containing insulin-producing cells in embryoid body outgrowth cultures with the use of dithizone. These cells when stimulated with glucose synthesized and secreted insulin in a glucose-regulated manner.
Conclusion: The data presented in this study showed that it is possible to generate insulin-producing cells from undifferentiated EC cells with the characteristics of pancreatic β cells.

Keywords: Embryonal Carcinoma Cells, Mouse Pancreas Extract, Insulin-producing Cells, Pancreatic β Cell Differentiation

Ps-28: Stem Cell Protein, Piwil2, Reprograms LNCaP Cell Line of Prostate Cancer to Cancer Stem Cell State

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Objective: Stem cell protein, Piwil2, as a member of argonaute gene family has been previously proved to be enriched in breast cancer stem cells (CSCs) population with high potential for tumorigenesis. To investigate the same role for this protein in prostate cancer as 2^nd rank cause of cancer death in men, we examined whether piwil2 overexpression can reprogram LNCaP cell line of prostate cancer to cancer stem cells state, enriching the cells with high level expression of putative biomarkers of cancer stem cells.
Materials and Methods: LNCaP cell line stably overexpressing piwil2 (piwil2-LNCaP) along with wild type LNCaP were cultured in RPMI medium containing 10% FBS, prior to being processed for total RNA extraction and subsequent cDNA preparation. Semi-quantitative and real-time PCR analysis were performed for both cell lines to evaluate the expression of CSCs biomarkers.
Results: Putative prostate cancer stem cell biomarkers were up-regulated in piwil2-LNCaP cell line, including BMI1, Sca1, and CD133.
Conclusion: The data demonstrated that Piwil2 could reprogram prostate cancer cell line to cancer stem cell state, hence introducing piwil2 as a promising biomarker for distinguishing prostate CSCs along with its applicability for therapeutics purposes.

Keywords: Piwil2, LNCaP, Prostate Cancer Stem Cells

Ps-29: Co-Expression of *OCT4* and Piwil2 Inclines Mouse Embryonic Fibroblasts to Pluripotency State

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Objective: Induced pluripotent stem (iPS) cells are the result of reprogramming differentiated adult cells utilizing four transcription factors, namely OCT4, SOX2, c-Myc and KLF4. As the aforementioned genes are classified as oncogenes, it seems to be a safer option to carry out the reprogramming without these oncogenes. There have been many attempts to replace them. For instance, our research team has previously suggested that the reprogramming of mouse embryonic fibroblasts (MEF) into a cancer stem cell state can be achieved through the ectopic expression of stem cell protein Piwil2 which is a member of argonaute protein family. After establishing MEF-Piwil2 cells, we thus further explored the possibility of introducing OCT4 as a pivotal pluripotency gene to these cells in order to enhance their pluripotency characteristics.
Materials and Methods: MEF-Piwil2 cells were transfected with a plasmid containing OCT4 gene that was under the control of CMV promoter via Neon Electroporation method. Subsequently, selection of the successfully transfected cells was carried out so that molecular analysis of the gene expression profiles of the cells could be performed using RT and real-time PCR.
Results: According to the results of RT and real-time PCR, in comparison with MEF and MEF-Piwil2, an increase was detected in endogenous pluripotency factors in MEF-Piwil2-CT4 cells. Moreover, it was revealed that the variant 2 of OCT4 emerged in MEF-Piwil2-OCT4 cells which is a characteristic of mouse embryonic stem cells and is normally absent in MEF-Piwil2 and MEFs. Consistent with MEF-Piwil2-OCT4 cells' transition from cancerous to pluripotency state, a significant reduction in their growth rate was also observed compared to that of MEF-Piwil2 with scored doubling times of 16h and 10h, respectively.
Conclusion: It is suggested that ectopic expression of Piwil2 in tandem with *OCT4* in MEFs can alter the transcriptional pathways of the cells, thus changing their fate and reprogram them back to a pluripotency state.

Keywords: *Oct4*, Piwil2, Pluripotency, Mouse Embryonic Fi-

broblasts

Ps-30: Choroid Plexus Epithelial Cells Induce Dopaminergic Differentiation of Human Adipose-derived Stem Cells

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Objective: Cell replacement therapy (CRT) is considered a promising approach for treatment of neurodegenerative disorders such as Parkinson's disease. Multipotent mesenchymal stem cells derived from adipose tissue exhibit plasticity in their differentiation into non-mesodermal lineages including neural cells. Choroid plexus epithelial cells (CPECs) form the primary part of blood- cerebrospinal fluid (CSF) barrier. CPECs possess the capacity for biosynthesis of various neurotrophic factors. In the present study, the cocktail of CPECs-conditioned medium (CPEC-CM) and knock-out serum (KoS) were used to induce dopaminergic differentiation of human adipose derived stem cells (hADSCs).

Materials and Methods: The subcutaneous adipose tissue was obtained during abdominal surgery in non-cancer patients and hADSCs were isolated and characterized by flow cytometry for the expression of mesenchymal stem cell markers. CPECs were cultured and examined for expression of transthyretin. Next, conditioned medium from growing CPECs were collected. The cells were then treated with CPEC-CM and KoS for 10 days. Reverse transcriptase polymerase chain reaction (RT-PCR), quantitative real-time PCR (qPCR) and immunocytochemistry techniques were performed to measure the efficacy of the neuronal differentiation.

Results: Human ADSCs exhibited remarkable morphological changes following CSF/KoSR treatment. The spindle-shaped hADSCs underwent cytoplasmic retraction, forming a contracted cell body with multipolar processes similar to long axon of neurons. Flow cytometric analysis showed that ADSCs were positive for CD73 and CD105, while negative for CD45. As revealed by RT-PCR, qRT-PCR and immunocytochemistry analyses, CSF/KoSR treated hADSCs expressed high levels of neuronal and dopaminergic markers Nestin, DAT, Nurr1, Pitx3 and TH.

Conclusion: This study provides a platform for further investigations regarding active integration of induced hADSCs, functionality of engrafted cells and disease modelling for neuronal therapies.

Keywords: Mesenchymal Stem Cells, Differentiation, Dopaminergic Neurons

Ps-31: Assessment of The Regeneration Potential of Cryopreserved Human Adipose Derived Stem Cells Conditioned Media to Skin lesions

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Objective: The use of adipose derived stem cells (ADSC) in regenerative medicine is in rise due to their plasticity, their capacity of differentiation, their paracrine and trophic effects. Our objective is to investigate the effect of cryopreservation of ADSC on their secretion and migratory capacity in vitro and in vivo.

Materials and Methods: We used wound healing assay to assess the in vitro migration capacity of cryopreserved ADSC and elisa to study the expression of protein secretion in the conditioned media. We also examined the effect of CM on cutaneous wound healing in rat excisional cutaneous wound module.

Results: Our results showed that CM stimulate the migration of ADSC, 24 hours after treatment (P=0.0067), this CM contains HGF, FGF and TIMP3. 42 days after in vivo injection, the histological analysis show that the thickness of the dermis become very close to the normal thickness (0.86 mm versus 1.46 mm; p value 0.016), a reduction in scar size) 1.18 mm versus 4.81 mm; P<0.0001), appearance of hair in the wound region (87.5% vs. 33.3%), improvement of the morphological, orientation, thickness and the density of collagen. The overall assessment of the scar was 8.37 versus 6.22; P<0.0001).

Conclusion: These results suggest that conditioned media (CM) of cryopreserved human adipose derived stem cells stimulate regeneration of cutaneous wound and accelerate healing.

Keywords: Conditioned Medium, Cryopreserved Human Adipose Derived Stem Cells, Wound, Regeneration

Ps-32: Assessment of Regenerative Wound Potential of Conditioned Medium Obtained from Long-Term Cryopreserved Human Adipose Derived Stem Cells

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Objective: The use of adipose derived stem cells (ADSC) in regenerative medicine is in rise due to their plasticity, their capacity of differentiation, their paracrine and trophic effects. Our objective is to investigate the effect of cryopreservation on ADSC paracrine secretion.

Materials and Methods: In this study, the effect of conditioned media (CM) of cryopreserved human adipose derived stem cells (ADSCs) on the migration and the paracrine secretion were evaluated in vitro. We examined the effect of CM on cutaneous wound healing in rat excisional cutaneous wound module.

Results: Our results show that CM stimulate the migration

of ADSC, 24 hours after treatment (P value =0.0067), this CM contains HGF, FGF and TIMP3. 42 days after in vivo injection, the histological analysis show that the thickness of the dermis become very close to the normal thickness (0. .86 mm versus 1.46 mm p value 0.016), a reduction in scar size) 1.18 mm versus 4.81 mm P value <0.0001), appearance of hair in the wound region (87.5% versus 33.3%), improvement of the morphological, orientation, thickness and the density of collagen. The overall assessment of the scar was 8.37 versus 6.22 P value <0.0001).

Conclusion: This results suggest that conditioned media (CM) of cryopreserved human adipose derived stem cells stimulates regeneration of cutaneous wound healing.

Keywords: Conditioned Medium, Cryopreserved Human Adipose Derived Stem Cells, Wound, Regeneration

Ps-33: The Impact of Early Subcultures on Gene Expression Profile and Resistance to Some Toxic Conditions in Human Adipose Tissue-Derived Stem Cells

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Objective: Adipose tissue-derived stem cells (ADSCs) are capable of multipotential differentiation and express several angiogenic, anti-apoptotic and immunomodulatory markers. These features make adipose tissue as a promising source of stem cells for regenerative medicine. However, for efficient translational use, culture-induced changes in the gene expression profile and resistance of the ADSCs to ischemic environment should be taken into consideration.

Materials and Methods: We compared the expression of some clinically important markers between the unpassaged and third-passaged ADSCs by Reverse transcription-polymerase chain reaction (RT-PCR), Quantitative real-time PCR (qPCR) and flow cytometry. Sensitivity of the ADSCs to oxidative stress, hypoxia and serum deprivation was also investigated.

Results: Embryonic stem cell (ESC)-specific markers were expressed in the unpassaged ADSCs but were downregulated after three passages. The expression of stemness-related genes, transforming growth factor B (TGFB) and fibroblast growth factor 2 (FGF2), was upregulated while FGF4 and leukemia inhibitory factor (LIF) were downregulated after three passages. The expression of angiogenic genes in the third-passaged ADSCs was higher than the unpassaged cells. Epithelial-mesenchymal transition (EMT) markers were either expressed in the third-passaged ADSCs or significantly upregulated after three passages. In contrast, cell cycle inhibitors, cyclin- dependent kinase inhibitore 1A (CDKN1A) and tumor protein p53 (TP53), were downregulated with early subcultures. The unpassaged and third-passaged ADSCs showed nearly similar resistance to oxidative stress, hypoxia and serum deprivation.

Conclusion: The present study confirms that the primary cultures of human adipose tissue contain a subpopulation of ESC-like cells, but the expression of pluripotency markers subsides rapidly in standard mesenchymal stem cell culture medium. The expression of angiogenic and EMT markers also varies with early subcultures. Altogether, third to fourth-passaged ADSCs may be better choices for transplantation therapy of injured tissues, especially after ischemic conditions.

Keywords: Subculture, ADSC, Hypoxia, Oxidative Stress, Serum

Ps-34: Oxytocin and Relaxin Induces Differentiation of ADSCs to Cardiomyocytes

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Objective: Oxytocin and relaxin are two hormones which are produced and secreted in heart and have known roles in the development of cardiovascular system and repair of ischemic heart injury. In the current study, we examined the effectiveness of these two hormones for cardiac differentiation of mouse adipose tissue-derived stem cells (ADSCs).

Materials and Methods: ADSCs were isolated from the inguinal fat pad of 8-10 weeks old mice and were characterized by flow cytometry. For cardiac differentiation, the ADSCs were cultured in complete medium and were treated with different concentrations of oxytocin or relaxin during the first 4 days. Three weeks after cardiac induction, the expression of cardiac-specific genes and proteins were examined by RT-PCR, qPCR and western blot.

Results: Differentiated ADSCs expressed cardiac transcription factors, Gata4, Mef2c, Nkx2.5 and Tbx5, and cardiac-specific genes, α-Mhc, β-Mhc, Mlc2a, Mlc2v and Anf. Based on qPCR analysis, 10-7 M oxytocin and 50 ng/ml relaxin upregulated the expression of MLC2a and MLC2v mRNAs. The expression of α-actinin, desmin and connexin43 proteins was also increased after treatment with 10-7 M oxytocin and 50 ng/ml relaxin.

Conclusion: These results indicated that oxytocin and relaxin improve cardiac differentiation of ADSCs. Both hormones seems to play significant roles in cardiac development.

Keywords: ADSC, Differentiation, Cardiomyocyte, Oxytocin, Relaxin

Ps-35: Predicting Targeting of Yamanaka's Factors by MicroProteins

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Objective: Gene expression requires assembly and identification of promoters by protein complexes. These proteins are established through protein-protein interactions. Any factor that disrupt or interfere with protein complexes activity or formation could affect gene expression. MicroProteins (miPs), are a class of newly identified small proteins that interfere in the formation of many protein complexes. These single domain proteins therefore would be able to regulate many biological functions, including gene expression. Con-

sidering the importance of four Yamanaka's factors (cMyc, Sox2, Klf4 and Oct3/4) in generation of iPS cells, we have predicted potential miPs that targeting these factors. Such study could help us better understand and manipulate the regulation of gene expression in the resultant stem cells.
Materials and Methods: The protein sequences of homo sapience both valid sequences and translated mRNA sequences were obtained from UniProtKB alongside the sequences of the four Yamanakas' factors. All protein domains were obtained from Pfam website. Then using local BLASTp program, we have identified those proteins with the following criteria as potential miPs. The predicted miPs should be smaller than 500aa in size, should have high level of identity with the proteins they are intended to target and most importantly should not have the DNA binding domains (DBD), which is present in their counterpart proteins.
Results: Our search resulted in identification of 299 such miPs in the human genome. Interestingly, most of miPs are targeting Klf4 (247 predicted miPs), while Sox2 had the lowest miPs targeting it (only 9 were predicted). Twenty-five miPs were found for Oct3/4 and 18 were predicted for Myc.
Conclusion: These findings indicate that all genes regulated by these four factors could be subjected to another level of gene regulation via miPs that interfere with the function of these factors.
Keywords: MicroProteins, Yamanaka's Factors, IPS Cells, Protein-Protein Interactions, Gene Regulation

Ps-36: Comparative Karyotype Analysis of Two Freshwater Planarian Species: Schimidtea Mediterranea and Dugesia Iranica

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Objective: Nowadays, regenerative medicine is the principle key in biology because of different diseases, infection, injury, damage or inoperative issue. Having an almost unlimited capacity to regenerate tissues lost to age and injury, planarians have long fascinated naturalists, whereas regeneration in human is limited. Freshwater Planarians are bilaterally symmetrical, and complex metazoans that repair damage to their bodies and cease remodeling when a correct anatomy has been achieved. Planarians are well known for their regenerative abilities and less well known is how planarians maintain spatial patterning in long-lived adult animals or how they re-pattern tissues during regeneration
Materials and Methods: karyology.
Results: We will briefly introduce planarians, explain two case studies of asexual populations of freshwater belonging to the genera Schmidtea mediterranea and Dugesia iranica, show, and compare karyotypes and morphology in these fascinating organisms. Cytogenetic studies were organized in freshwater planarians with samples of Schmidtea and Dugesia, which are collected from Gachsar, Iran.
Conclusion: Iranian planarians' karyology has studied for the first time in this article and compare with Schimidtea mediterranea, as the famous model in the word. The first observation shows that both genera are different in their karyotypes and phenotypes and it can help our research to talk more about different power of regeneration in planarians.
Keywords: Planarians, Morphology, Karyology, Chromosomes

Ps-37: Finding Regulatory Elements in Direct Conversion of Fibroblast to Cardiomyocytes Using Chemical Cocktails

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Objective: Direct conversion of fibroblast to cardiomyocytes has been successfully achieved using chemical cocktails by several groups. In this approach, conventional overexpression strategies have been replaced by more effective and less manipulative approach. Genes expression changes during this conversion have been studied, albeit not in details. Unfortunately, transcription factors(TFs) and miRNAs that are involved in this process are unknown. In the current study, we have used Bioinformatics approaches to identify the most important TFs and miRNAs involved in this conversion.
Materials and Methods: We have combined the gene expression data obtained from 8 independent reports on direct conversion of fibroblast to cardiomyocytes, which include 141 data sets. Using online tools, freely available software and in-house scripts, we have compared the gene expression of fibroblast with those of cardiomyocytes and iPS driven cardiomyocytes (iCMs) individually. In addition, several interaction networks of involved factors were also constructed and analyzed.
Results: We have identified the most important differentially expressed TFs in the process of developing iCMs from fibroblasts. Besides, we have introduced the miRNAs with a role in this process. The constructed network shows dynamic changes in the regulatory elements during this conversion.
Conclusion: Our study could be helpful in designing a more targeted approach for developing iCMs using alternative chemicals with known impacts at the molecular level. And as whole, would be useful in safer treatment of heart failure.
Keywords: Direct Conversion, iPS Driven Cardiomyocytes (iCMs), Chemical Cocktails, Fibroblasts, Regulatory Network

Ps-38: Acellular Dermal Matrix Modulates Adipose Derived Stem Cells toward A Low Fibrogenic Phenotype

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Objective: Pressure, venous, and diabetic ulcers contrib-

ute to increased morbidity and mortality in affected people and impose significant financial burdens on healthcare systems. For chronic wounds coverage, acellular dermal matrix (ADM) as an extracellular matrix (ECM)- based biomaterial has many advantages over synthetic polymer materials, including significant mechanical strength and retained biological activity. Further, repopulating cells into the ECM scaffold before implantation may help the graft to restore its function. The use of adipose tissue-derived stem cells (ADSCs) is a promising treatment because of the relatively non-invasive autologous stem cells extraction, and there is growing evidence that shows ADSCs release cytokines and growth factors that promote wound healing.

Materials and Methods: The aim of our study was to develop an ADSCs-populated ADM and assess its characteristics *in vitro* with the goal of promoting of scar-free wound healing. We used decellularized mouse skin to prepare ADM and then seeded human ADSCs on this scaffold and compared it to a regular 2D culture at different time points. Combinations of surface markers and intracellular proteins were used to characterize cultured cells.

Results: The results showed that although the traditionally accepted markers for ADSCs (i.e., CD73, CD44 and CD 90) were well preserved in 2D cultures compared to ADM culture, high expression of procollagen and α-smooth muscle actin (α-SMA) in 2D cultured cell suggest these are mainly myofibroblastic differentiated cells rather than progenitors. Additionally we found a significant retention of expression in CD34, CD45, CD31, and haematopoietic and endothelial cell markers, in ADSCs cultured on ADM compared to 2D culture.

Conclusion: These data suggest that adipose derived cells keep their progenitor phenotype more potently in ADM culture compared to 2D culture and that ADSC differentiation into myofibroblasts can be regulated by culturing them on ADM. This makes ASC-ADM combination as a therapeutic approach to reduce the risk of fibrosis upon transplantation of cell populated ADM, while having the benefit of natural extracellular matrix wound coverage and ADSCs to promote wound healing.

Keywords: Wound Healing, Adipose Tissue-derived Stem Cells, Acellular Dermal Matrix

Ps-39: Evaluation of Effect PRP in Gene Expression of The Sox-9, Aggrecan, Comp and Type Ii Collagen in Chondrocyte Cells Differentiated from of Wharton Jelly Stem Cells

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Objective: Osteoarthritis (OA) is the most common multifactorial joint degrading disorder which severely affects the life quality of elder people. Due to limited capacity of chondrocyte for proliferation, nowadays, mesenchymal stem cells are introduced for cell-based therapies in OA. Platelet rich plasma (PRP) is another factor capable to promote cell proliferation and finally heal cartilage defects. The goal of this study is to evaluate whether PRP can induce Wharton's jelly derived mesenchymal stem cells into chondrocytes.

Materials and Methods: Mesenchymal stem cells were isolated using Wharton's jelly explants method. Isolated cells were divided into control and PRP-treated and incubated for 2 weeks. Then the expression of cartilage specific genes such as collagen II and Sox-9 was evaluated.

Results: Wharton's jelly derived stem cells were capable to differentiate into chondrocyte in the presence of PRP and also PRP-treatment resulted in promotion of collagen II and Sox-9 gene expression.

Conclusion: PRP can differentiate Wharton's jelly derived stem cells into chondrocyte and can be introduced as a promoting factor for OA cell-therapy.

Keywords: Mesenchymal Stem Cells, Wharton's Jelly, Platelet Rich Plasma, Osteoarthritis

Ps-40: The Measurement of Histone Marks as A Rapid Assessment of Cell Pluripotency

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Objective: Histone H3 lysine 9 methylation (H3K9me) has been shown as a critical barrier to efficient cell reprogramming both following induced pluripotent stem cell generation and somatic cell nuclear transfer. These discoveries allow for the development of specific approaches to assess the cell pluripotency state by considering the extent of H3K9 methylation compared to a competing acetylation at the same position.

Materials and Methods: A set of pluripotent and differentiated human cells including ES and iPS cells, along with human fibroblasts and their derived reprogrammed cells, were used to evaluate the ratio of total H3K9 methylation over acetylation using a quantitative ELISA-based approach. Additionally, using ChIP-qPCR the occurrence of these histone marks on the regulatory regions of several stemness genes (Nanog, Oct4 and Sox2) as well as on genes indicating fibroblast differentiation (Vim, COL1A1 and THY1) was evaluated.

Results: Results showed remarkably high ratios of H3K9ac/K9me2 in ES cells and in reprogrammed pluripotent cells and very low ratios of the same marks in differentiated cells. In pluripotent cells, a direct relationship between the ratios of total H3K9ac/H3K9me2 and the ratios of these marks on pluripotency gene regulatory regions and their expression was observed. In differentiated cells, in contrast, the ratios of global H3K9ac/K9me2 is low but in contrast to pluripotent cells, in differentiated cells, the active gene promoters do not follow the general situation of H3K9 modifications.

Conclusion: Based on the data presented here, we propose that the rapid quantitative measurements of relative amounts of H3K9ac and K9me2 in iPS cells compared to the parental differentiated cells constitute a reliable and convenient criterion to rapidly assess the cell pluripotency potentials and the efficiency of cell reprogramming.
Keywords: Pluripotency, ESC, iPSC, Histone Mark

Ps-41: Evaluation of Inhibitory Effect of Silibinin on Breast Cancer Stem Cells

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Objective: Breast cancer stem cells have been introduced as a cancer resistant factor to current conventional therapies that cause relapse and metastasis. Silibinin is extracted from the milk thistle seeds and has been shown to exert the anti-proliferation effects, apoptosis induction, resulting in blocked tumorogenesis. The aim of this study was to find out the capability of Silibinin as an herbal therapeutic for targeting cancer stem cells.
Materials and Methods: Mamospheres were generated from MCF-7 cell line in non-adherent and serum-free medium. The Silibinin effect was then evaluated on stemness properties; including expression of stemness markers, colony-forming ability, invasion and growth of these spheres.
Results: Our results showed that 1500 μM/ml of Silibinin is effective on cell viability through 0.2 and 0.3 fold down-regulation of BCL2 and SURVIVIN genes, respectively. Moreover, it reduces 0.2, 0.4. 0.2, 0.09 and 0.5-fold expression levels of CD133, ALDH, C-MYC, NANOG and SOX2 genes respectively, which are stemness-related factors and also reduces 15 and 5-fold colony and sphere forming ability through affecting breast cancer stem cells derived spheres.
Conclusion: Silibinin as an herbal medicine can affect breast cancer stem cells and cause stemness properties inhibition, cell growth and invasion ability *in vitro* with the least side effects.
Keywords: Cancer Stem Cells, Breast Cancer, Silibinin, Stemness

Ps-42: The Effect of Lifestyle on The Mode of Delivery Among Girls in Lar

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Objective: Delivery and its dangers play an important role in choosing mode of delivery. Since today's young girls will be future mothers, their outlook determines their type of delivery in the future. This study addressed their views in order to provide logical and helpful solutions in this area.
Materials and Methods: This cross-sectional study was conducted in a specific time period. The sample included 138 high school girls in Lar who were selected using cluster random sampling from different regions. Their opinions were collected using open questions (regarding choice of delivery mode and the reasons). Data were analyzed using descriptive and analytic statistics (correlation coefficient, t test, ...).
Results: Data show that 117 (84.8%) young girls would choose delivery via caesarean. The reasons for this choice include lifestyle common in the region, experience of their mothers and close relatives during childbirth, and statements by their friends regarding lack of emotional support for natural delivery. There was a significant correlation between the mode of delivery of mothers and the choice of their girls ($f = 32.9$, $P < 0.05$).
Conclusion: The results show that, in the not too distant future, there will be an observed increase in Caesarean cases, mostly because of descriptions the girls hear as well as their observation of other women' experience. though there has been much propagation aiming at increasing natural delivery, the necessary measures have not been taken. Evidence shows that still non_expert people are involved in natural childbirth, and often use inaccurate and sometimes painful procedures. These bitter experiences are transferred to today's girls and they prefer cesarean delivery under expert supervision. Training delivery staff and using professional and compassionate people in moments of painful labor, who are empathetic to mothers, will best promote natural delivery methods.
Keywords: Lifestyle, Delivery, Girls, Lar

Ps-43: Enhanced Differentiation of Mesenchymal Stem Cells Cultured on Gelatin Scaffold

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Objective: All cells in solid tissues are surrounded by a highly dynamic 3 dimensional (3D) structure composed of proteins and polysaccharides, defined as extracellular matrix (ECM). ECM provides structural support for cells, and serves critical functions in controlling cell behavior and directing cell fate and function. Many studies have been focused on the research of biocompatible scaffolds which provides 3D structure that mimics the natural ECM to improve stem cell proliferation and differentiation. The purpose of the present study was to investigate the *in vitro* efficacy of the gelatin cryogel scaffold to induce hepatocyte differentiation of human adipose derived mesenchymal stem cells.
Materials and Methods: The third passage of mesenchymal stem cells isolated from human adipose tissue were seeded in the polystyrene plates and gelatin cryogel disks with 160-180 nm diameter. Hepatogenic differentiation was induced using hepatocyte growth factor (20 ng/ml), Dexamethasone (10-7M)

and Oncostatin M (10 ng/ml) during 21 days. The efficiency of hepatocyte differentiation in the 3D scaffold was characterized using morphological, biochemical (urea production and glycogen storage) and molecular (albumin, alpha fetoprotein, CK 18 and 19 RNA expression analysis) experiments. Mesenchymal stem cells and HepG2 cell lines used as negative and positive controls respectively.
Results: Evaluation of morphological changes, glycogen storage, and secretion of urea and expression of hepatocyte specific genes in 3D differentiated hepatocyte-like cells showed improvement (P value < 0.05) when compared with the control group and those cell differentiated on the polystyrene.
Conclusion: Our findings indicate that 3D gelatin scaffold significantly improves the hepatic differentiation of adipose derived mesenchymal stem cells and could have been considered for cell therapy of liver and liver tissue engineering.
Keywords: Mesenchymal Stem Cell, Gelatin, Three Dimensional Scaffold, Hepatocyte

Ps-44: Assessment of Caspases 3/7 Activity during Human Embryonic Stem Cell Differentiation into The Cardiomyocyte

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Objective: Although the cell differentiation is an inseparable part of development in multicellular organisms, the regulating molecular pathway of it is still not fully defined. In the other hand, the process of programmed cell death, or apoptosis, which is generally characterized by a series of distinct morphological features and energy-dependent biochemical mechanisms, notably, has shown several common features with cell differentiation such as caspases activation, cyochrom c release and apoptosome complex formation. The goal of this study is the assessment of the role of the caspases 3/7 activation during normal differentiation of human embryonic stem cell (hESCs) into the beating cardiomyocytes.
Materials and Methods: Caspases 3/7 activity as the final fate of mitochondrial pathway of cell death were investigated during differentiation of hESCs into beating cardimyocytes.
Results: Caspases 3/7 show a reduced and gradually elevated level of activity during differentiation comparing with apoptosis process. It seems that the time of engagement and the activity level of caspases 3/7 may contribute to the hESCs differentiation.
Conclusion: It has been sujested that caspases 3/7 are activated during differentiation and that this activation is apparently required for the normal human embryonic stem cell differentiation into the beating cardiomyocyte
Keywords: Mitochondrial mediated apoptosis, Apoptosom complex, Cytochrom c release, Caspases activity

Ps-45: Investigating The Expression of Tyrosine Hydroxylase Gene in The Newborn Rat Brains Influenced by Linseed Oil

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Objective: During the pregnancy, many factors influence the development of the fetus including maternal nutritional factors. Linseed oil is one of the vegetable oils containing unsaturated fatty acids and other compounds having different effects on gene regulation and speed cellular messaging. The early fluctuations in gene expression related to the brain development leads to the long-term effects.
Materials and Methods: In this research, the effect of linseed oil on Tyrosine hydroxylase gene expression was investigated in developing rat brains. Fifteen pregnant female rats were distributed into 3 groups of 5 each including control, low dose and high dose. The control group had no treatment. The pregnant rats in the low dose group received 1 g/Kg/BWt linseed oil and the high dose group received 5 g/Kg/BWt linseed oil via gavage respectively. After giving birth, the maternal behavioral tests were performed and the data was recorded. Several newborn rats selected to determine the effect of linseed oil on the expression of tyrosine hydroxylase gene using Real-time PCR method.
Results: Real-time PCR data analysis indicated that as the linseed oil given to the mothers was increased the expression of tyrosine hydroxylase gene in the newborn rat brains was significantly reduced.
Conclusion: The results of this study indicated that linseed oil might affect brain development through its impact on effective neural gene expressions.
Keywords: Linseed Oil, Brain Development, Gene Expression

Ps-46: Human Amniotic Fluid Stem Cell Therapy Effects' on Ovarian Cancer Apoptotic Genes by Co-cultured with SKOV3

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Objective: Human amniotic fluid stem cells (hAFSCs) have two critical stem cells features': self-renew and differentiation potential. Unique properties of hAFSCs are safety obtaining method, easy and fast isolation, no ethical problem, low immunogenicity, and natural tumor tropism. During last decades scientists follow the low cost, and accurate treat-

ment method for cancers. One of the most common women malignancies with problematic diagnosis, and great mortality rate is ovarian cancer. Thus, the goal of our experiment is confirming that hAFSCs can be an efficient and safe therapeutic tool for ovarian cancer patients.

Materials and Methods: In this study, we obtained 5 ml amniotic fluid from pregnant women through amniocentesis in Al-Zahra hospital of medical university Tabriz-Iran. HAFSCs were isolated from all samples, and characterized then co-cultured with SKOV3 (ovarian cancer cell line), using transwells in 24 wells plate. Human skin fibroblast cells (hSFCs) were used as a negative control. After 5 days, SKOV3 and hSFCs viability were assessed with MTT assay and P53, P21 as apoptotic genes were investigated by real time PCR.

Results: We successfully isolated, characterized and established hAFS cell lines. After 5 days of co-culture hAFSCs with SKOV3 and hSFCs, cells viability of SKOV3 were decreased but at hSFCs were not shown toxicity by MTT assay. P53, P21 genes of SKOV3 have down regulation and significantly low expression by real time PCR compared with hSFCs. (P-value<0.05)

Conclusion: Our findings showed that hAFSCs have natural tumor tropism, and release soluble factors in cell culture, so they show efficient anticancer effect. Thus, in the future we can use hAFSCs for anticancer targeted therapy on ovarian cancer *in vivo*.

Keywords: Human Amniotic Fluid Stem Cells (hAFSCs), SKOV3, Apoptotic Genes, Co-Culture, Anticancer Therapy

Ps-47: Efficient Banking of Human Amniotic Fluid Stem Cell Lines

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Objective: Amniotic fluid-derived stem cells (AFSC) with self-renewal and pluripotential capability become a critical and safe biomaterial without any ethic issue for using in cell therapy and tissue engineering. Human AFSCs can be gained from 2-5 mL of amniotic fluid through diagnostic amniocentesis for prenatal screening. For sufficient use of hAFSCs in clinic, we optimized banking system for storage these cells for long time with preserve their unique properties.

Materials and Methods: 5ml of amniotic fluid was obtained through diagnostic amniocentesis in 2th trimester after singing written consent form by donors. HAFSCs were isolated, characterized well by FACS and RT-PCR. There protocols were investigated for freeze/thaw cycle (Respectively protocol 1(5%), 2(10%) and 3(15%) DMSO). Pluripotential capability of hAFSCs were evaluated with expression of NANOG gene by real time PCR and cell viability were assessed by MTT assay and real time PCR for cell cycle genes and growth pattern in cell culture by inverted microscope before and after the 3 protocols of freeze/thaw cycle.

Results: This study indicated that protocol 2 with 10% DMSO was better than other protocols such as: was shown the most proliferation and attachment of AFSCs by inverted microscope, the most significant cell viability among 3 protocols and the most significant expression of NANOG gene.

Conclusion: The findings of our study show that protocol 2 performed significantly results on characterized cell lines and cell viability. This protocol has the least cytotoxicity on hAFSCs with preserve their self-renew and pluripotential capacity and it is useful freeze/thaw cycle for AFSC lines banking for long time.

Keywords: Banking, Cell Viability, Freeze/Thaw Cycle

Ps-48: Human Umbilical Cord Wharton's Jelly Stem Cells (HUCWJSCs) Can Differentiate into Keratinocytes

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Objective: Stem cells are undifferentiated cells and found in different tissues. These cells have the capacity of self-renewal and differentiation into other lineages that termed potency. Based on potency they are classified as: totipotent, pluripotent, multipotent, and unipotent. Human umbilical cord Wharton's jelly stem cells (HUCWJSCs) are the multipotent stem cells that studied in regenerative medicine. Differentiation potential of HUCWJSCs previously investigated and showed that these cells can be differentiated into other cell lines such as lens fiber cell, chondrocytes, neurons and osteoblasts.

Materials and Methods: HUCWJSCs were isolated from human umbilical cord Wharton's jelly by explant culture and then were proliferate in T25 flasks. In the fifth passage, the expression of mesenchymal stem cell markers (SC017) were assessed by flowcytometry. In experimental groups HUCWJSCs were induced by keratinocyte differentiation inducer (sc-311368) and expression of keratin14 (Ab175549) were investigated with western blotting technique.

Results: Flowcytometric analysis showed 44.08% Stro-1, 26.84% CD90, 48.22% CD105, 57.49% CD44, 25.51% CD45, 43.25% CD146, 13.94% CD106, 10.88% CD166, 22.78% CD19 expression in HUCWJSCs. Differentiated cells expressed keratin14.

Conclusion: HUCWJSCs have mesenchymal stem cell markers that can be differentiated into keratinocyte as well as lens fiber cells, osteocytes, chondrocytes and neurons.

Keywords: HUCWJSCs, Flowcytometry, Western blotting, Keratin14

Ps-49: Overexpression of Piwil2 Induces Cancer Stem Cells Properties In MCF7 Breast Cancer Cell Line

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Objective: Piwil-like RNA-mediated gene silencing 2 (Piwil2) is a member of AGO/PIWI gene family which is enriched in cancer stem cells. Being highly expressed in different types of cancer, it can contribute to biological pathways such as the epithelial-to-mesenchymal transition (EMT) where the loss of cell-cell adhesion leads to a shift in the cytoskeletal dynamics of cells by changing the epithelial morphology of the cells to a mesenchymal phenotype. EMT is the main factor responsible for the progression of the cancer. The objective of this research was to investigate the overexpression of Piwil2 and its role in the induction of EMT and cancer stem cells properties in MCF7 breast cancer cell line.
Materials and Methods: MCF7 cells were transfected with a plasmid containing human Piwil2 that was under the control of CMV promoter utilizing Neon Electroporation method. Subsequently, selection was carried out using G418 and doubling time was calculated in the transformed and control cells. RT and real-time PCR were also performed to analyze the expression of epithelial and mesenchymal genes as well as those related to cancer stem cells.
Results: The molecular analyses revealed a significant reduction in the expression of epithelial genes (Ovol-2, E-cadherin and Zo-1) while a significant increase was detected in the expression of mesenchymal markers (Vimentin, Zeb-2, Slug, Snail and Fibronectin) as well as a few cancer stem cell biomarkers including Oct4, CD44 and ALDH.
Conclusion: Based on the results from this study it can be proposed that the ectopic expression of Piwil2 can trigger the process of EMT which loosens the tight extracellular connections of the cells rendering them invasive as they start to possess stem cell-like characteristics and adopt a mesenchymal morphology. Hence, Piwil2 protein seems to act as a master regulatory protein that is able to manipulate the transcription through specific signaling pathways which allow the cells to gain stem cell-like properties. Furthermore, it can participate in invasion and metastasis of tumor cells through activating the EMT process.

Keywords: Piwil2, Breast Cancer Stem Cells, Epithelial-to-Mesenchymal Transition, MCF7

Ps-50: Development of Large-Scale Manufacturing of Adipose-Derived Stromal Cells for Clinical Application

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Objective: *In vitro* expanded adipose-derived stromal cells (ASCs) are a useful resource for tissue regeneration. Translation of small-scale autologous cell production into large-scale production for allogeneic clinical applications necessitates wise selection of media and a cell culture platform to achieve a standardized and reproducible cell product. The greatest challenges of cell therapy manufacturing are to develop a scalable manufacturing process according to cGMP, while maintaining critical quality parameters. We have optimized clinical-grade culture conditions in the automated functionally closed hollow fiber bioreactor Quantum Cell Expansion System in pursuit of safe and feasible large-scale production of ASCs.
Materials and Methods: Stromal vascular fraction (SVF) was isolated from abdominal fat; ASCs were culture expanded over two passages in α-MEM with either 10% fetal bovine serum (FBS) or 5% human platelet lysate (hPL) in bioreactors coated with cryoprecipitate. Metabolic monitoring guided feeding rate and time of harvest. Viability, sterility, purity, differentiation capacity, genomic stability and cryopreservation of ASCs were examined.
Results: Each passage of this two-passage process demonstrated that hPL was superior to FBS with regard to support of proliferation of ASCs. ASCs fulfil ISCT and IFATS criteria in both media types (immunophenotype and triple differentiation). Comparative genomic hybridization demonstrated genomic stability. Sterility, mycoplasma and endotoxin tests were all negative. Optimal cryopreservation of ASCs was investigated and found feasible for therapeutic use.
Conclusion: The Quantum Cell Expansion System provides a reliable and efficient mean of manufacturing ASCs while minimising manipulation, labour-intensive manual tasks and risk of contamination. hPL serves as an effective growth supplement for expansion of clinical-grade ASCs. They can be cryopreserved in proper way where they can be directly injected in the patients just after thawing. The cells exhibit no chromosomal aberrations and meet all release criteria, indicating a clinically-feasible option for manufacture of large-scale allogeneic off-the-shelf ASC products originating from a single donor.

Keywords: Adipose-Derived Stromal Cells, Cell Culture, Close Cultivation System, Cryopreservation, Clinical Application

Ps-51: A Novel Technique for Decellularization of Pancreas Scaffold; Ductal Versus Arterial Catheterization

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Objective: Type 1 diabetes is the most common disease which is due to the lack of β cells. Regenerative medicine approaches such as a bioartificial pancreas has been proposed as potential therapeutic solutions. Increasing pancreatic islet survival and function is a starting point for obtaining a valuable bioartificial pancreas. Here there is two novel decellularization technique that preserve tissue architecture in

addition to complete decellularization.
Materials and Methods: Forty rats were selected and divided into two groups. Twenty pancreas were harvested by cannulation of common bile duct. The second group was catheterized by aorta near the origin of superior mesenteric and celiac artery bifurcations. All the extracted scaffolds were washed by normal saline. Then different percentages of SDS-Triton based solution at different times points were applied. The decellularized samples by both method of cannulation were examined by histopathological evaluation, DNA quantification and collagen content.
Results: The Histopathological evaluation showed that perfusion base-decellularization of whole pancreas by pancreatic artery and ductal perfusion effectively removes cellular and nuclear material but can retaining innate molecular and spatial framework and stiffness with perfusable vasculature and appropriate extracellular matrix (ECM) component to mimic pancreatic cell composition.
Conclusion: The novel strategy to produce a decellularized pancreas by ductal and arterial access could have the road for pancreas tissue engineering to deliver the cell to both exocrine and endocrine part of the tissue. Also both methods could provide a 3D-engineered pancreas comparable to the native pancreas.
Keywords: Pancreas, Bioscaffold, Decellular

Ps-52: The Effect of Rosemary Extract on The Proliferation and Survival of Human Adipose Stem Cells

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Objective: Rosemary has long been used as a medicinal herb, however little is known about the effect of Rosemary extract (RE) on human adipose stem cells (HASCs). HASCs has more benefits than bone marrow stem cells, so that many stem cells were extracted from one gram of adipose tissue. Adipose tissue extraction is easier than bone marrow and with no pain. In this study, the effect of RE on HASCs proliferation and survival rate was investigated.
Materials and Methods: Peritoneal adipose samples were isolated from pregnant women over 25 years and HASCs were extracted by using mechanical and enzymatic digestion and cultured in α-MEM and serum 15% FBS. In order to ensure the purity, the fourth passage cells were examined by flow cytometry and adipocyte, osteocyte differentiation media. The rate of cell proliferation and survival were assessed at doses of 50,100 and 150mg/ml of RE in 24, 48 and 96 hours, with MTT and cell counting methods.
Results: More than 90% of cells were positive for CD105, CD90, CD73 markers and less than 2% of cell were positive for CD45 and CD34. The HASCs showed a high proliferation and survival rate at 50 mg/ml of RE for 48 hours of treatment.
Conclusion: With *in vitro* induction of RE, it will be prepared a large number of HASCs for transplantation to treat neurodegenerative diseases.
Keywords: Human Adipose Stem Cell, Rosemary Extract, Proliferation, Survival

Ps-53: Nanotopographic Matrix Can Facilitate The Interaction of Mir-125b By MEG3 during Osteogenic Differentiation of Mesenchymal Stem Cells in BMP Signaling Pathway

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Objective: Autograft has traditionally been the gold standard in bone grafting to regenerate the bone . However, it has problems such as the limited volume of bone grafts and potential donor site morbidity, the risk of wound infection and trauma. Given these problems the Third-generation bio- materials by using combinations of osteogenic progenitor cells, engineered materials, biomedical technology and bio- active factors are being created to stimulate the bone tissue , s specific environment and architecture. Aligned Electrospun nanofibers have been increased tremendously over the last decade because of its ability to generate fibers with a diameter ranging from nanometer to micrometer scales which is similar to identified extracellular matrix elasticity and topography in ECM proteins such as collagen. topographical surface features are detected by a variety of mechanosensors, which this resulted biochemical signalling cascades including bone morphogenetic protein (BMP)/transforming growth factor-β (TGF-b) and non -coding RNA are important regulatory elements which can lead to the regulation of specific transcription factors in osteogenic differentiation.. It seems that the investigation of the complex interplay between nano topographical cues niche and microRNA–lncRNA interactions in hMSCs is an effective tool to find the new strategy for enhancing the induction of osteogenesis. This is a serious challenge in the field of tissue engineering and has not been elucidated yet.
Materials and Methods: In this study ,based on microRNA microarray data analysis we prepared a list of the osteoblast specific miRNAs which their activities increased to promote several signalling pathways in osteogenic differentiation of MSC. Then,we identified putative target genes of these miRNAs by using several prediction computational algorithms such as TargetScan 6.2 ,PicTar, TarBase ,miRanda ,mir Base. Furthermore ,to investigate the microRNA–lncRNA interactions, we used several target prediction databases such as miRcode: a comprehensive searchable map of putative microRNA target sites across the complete GENCODE and DIANA-LncBase , NONCODE, lncRNAdb, C-It-Loci, LNCipedia 4.0. In the present study ,we selected the MEG3 as a novel long noncoding RNA in Osteogenic differentiation of MSC ..In addition, DIANA-mirPath v.2.0, miRWalk , DIANA-TarBase v6.0 algorithms were used to analyze the target genes for the candidate miRNAs involved in BMP signaling pathway.Then, Aligned and randomly oriented Ploy (L-lactide) PLLA scaffolds were fabricated via electrospinning.Human bone marrow and adipose tissue drived

MSC were isolated and seeded on the scaffolds with an initial cell density of 2×10^4 cells per cm^2 then incubated in the osteogenic medium for 21 days.The differential non coding RNAs expression profiles were established by microarray. Microarray data were validated using qRT-PCR. To assess the effect of nanotopography on miRNAs expression, the miRNA regulatory network in BMP singnaling pathway as unique inducers of osteogenic differentiation of hMSCs was explored. Furthermore; after 7,14 and 21days, capacity for the osteogenic commitment of MSC was evaluated by measuring ALPase activity, extracellular calcium deposition using Cresolphthalein complex one method and qRT-PCR evaluated changes in osteogenic marker gene expression triggered by topographical cues. In addition,to explore the correlation between up- regulated MEG3 and down- regulated post-transcriptional miR-125b, we analyzed the expression levels of both genes during the osteogenic differentiation of MSC

Results: These findings demonstrate that hMSC, cultured on electrospun PLLA aligned fibers, had higher ALP activity and calcium deposition in comparison with Tcps. Furthermore; it seems that the stem cell fate could be regulated by nanotopographical cues in two different ways: first,by inducing direct mechanotransduction and second, indirect mechanotransduction which are detected by a variety of mechanosensors, including integrins and influnced on global expression profiles of microRNA during the osteogenic differentiation and The regulation of lncRNA expression as key regulators of BMP signaling pathway. Thus, the aligned orientation of nanofibers enhanced mineralization along the longitudinal axis of nanofibers, which would provide a beneficial approach for bone regeneration.

Conclusion: Investigation of the geometry mechanics on differentiation of mesenchymal stem cells in order to Control abilities the proliferation and differentiation of stem cells is an attractive prospect for clinical applications. This research further illustrates the investigation of the effects of microRNA on lncRNAs to elucidate of the complex ncRNA regulatory during osteogenesis that seem to be an important tool for controlling stem cell fate in engineered tissues to develop appropriated scaffolds similar to tissue structure in nano dimensions and create an artificial niche for stem cells . We hope our results will facilitate understanding of the molecular mechanisms of osteogenic differentiation and enhance tissue-engineering strategies as tissue substitutes for future bone regenerating

Keywords: Long Noncoding RNA, MEG3, MicroRNA, Topography, Osteogenic Differentiation

Ps-54: Analyzing Genomic Integrity of Mouse Embryonic Stem Cells in Ground State Culture Conditions

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Objective: Pluripotent cells stand in a transient state during early development, and segregation and supplying these cells *in vitro* leads to their extension as embryonic stem cells (ESCs). Finding the optimum culture condition is a hot debate topic; many scientific groups are working on different cocktails of molecular factors to maintain the distinguished properties of Stem Cells: their self-renewal and their potential to differentiate into the three germ layers. There are currently three main culture conditions for mESCs each of which have their own advantages and shortcomings. The conventional condition using Serum and feeder cells can cause a high level of heterogeneity in the cultured cells. It has been reported that mouse pluripotent cells cultivated in 2i and R2i are in the ground state of pluripotency but Karyotyping tests indicate mESCs cultured in R2i have much fewer chromosomal abnormalities compared to 2i. Different culturing conditions, such as media composition, may affect the nature and the frequency of acquired genetic aberrations varying in size from point mutations, through copy number changes in small genomic elements (e.g. amplification of repetitive sequences and retroelement mobility), to large chromosomal aberrations, trisomies and monosomies. In this study we focused on measuring the genomic integrity of mESCs cultivated in Serum, 2i, and R2i to find out in which condition the cells are more stable from different aspects at the genomic level and also look into the probable underlying causes.

Materials and Methods: The activity of transposable elements (Line-1, IAP and major-Satellites) was measured by profiling their methylation pattern with Pyrosequencing and qRT-PCR. In addition the expression level of a group of selected genes involved in genomic integrity was analyzed with qRT-PCR. To assess DNA fragmentation of mESCs in all three condition two Complimentary methods including gamma-h2ax assay and comet assay were used.

Results: The results indicate a higher level of transposon activity and more DNA damage in mESCs cultivated in 2i in comparison to R2i.

Conclusion: Not inhibiting Gsk3-beta in R2i condition helps to make stable mES cells with stable genome.

Keywords: Genomic Instability, Comet Assay, DNA Methylation

Ps-55: The Role of Cancer Stem Cell on Tumor Relapcing

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Objective: Cancer stem cells (CSCs) lead to cancer extension by producing transient-amplifying cells. These cells have the power to self-renew and to form heterogeneous populations that create the tumor bulk.

Materials and Methods: Preclinical studies have displayed that CSCs intercede tumor metastasis and resistance to chemotherapy and radiotherapy because CSCs are known to retain a nonproliferative state and to enter the cell cycle only

randomly, with those remaining after riddance of bulk cancer cells potentially giving rise to disease relapse and metastasis when they reenter the cell cycle after a term of latency.
Results: Targeting of the shift between quiescence and proliferation in CSCs is a potential tactic for impeding the reinitiation of malignancy, emphasizing the importance of clarification of the mechanisms by which these cells are kept in the quiescent state. Intrinsic and extrinsic regulators are liable to the control of cell cycle progress in CSCs.
Conclusion: In this review, we consider the systems by which CSCs may resist medical therapy and lead to tumor relapse.
Keywords: Cancer Stem Cells, Tumor Relapse, Self-renew, Cell Cycle

Ps-56: A Osteoblast-Seeded Bioactive Glass/Gelatin Nano-Composite: a Promising Bone Substitute in Critical-Size Calvarial Defect Repair in Rat

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Objective: Amid the plethora of methods to repair critical bone defects, there is no one perfect approach. In this study, we sought to evaluate a potent three-dimensional (3D) bioactive SiO2-CaO-P2O5 Nano-Bioglass/Gelatin scaffold for its biocompatibility by seeding cells as well as for its regenerative properties by animal implantation.
Materials and Methods: Osteoblast cells were seeded onto nanocomposite scaffolds to investigate the process of critical-size calvarial defect via new bone formation. Scanning electron microscopy (SEM) was used to validate topography of the scaffolds and its homogeneity. Proliferation assay and confocal microscopy were used to evaluate its biocompatibility. To validate osteogenesis of the bioactive nanocomposite scaffolds, they were first implanted into rats and later, removed and analyzed at different time points post mortem using histological, immunohistochemical and histomorphometric methods.
Results: The results confirmed biocompatibility of the scaffold. Moreover, seeded scaffolds with osteoblasts enhanced repair of critical bone defects via osteogenesis.
Conclusion: We demonstrate the feasibility of engineering a nanocomposite scaffold with an architecture resembling the human bone, and provide proof-of-concept validation for our scaffold using a rat animal model. Our *in vivo* results indicate that our nanocomposite provides a significant contribution to bone regeneration and is highly biodegradable and biocompatible. Thus such a composite could have potential in bone regeneration and there is hope to use it as an efficient bone substitute alternative.
Keywords: Bone Tissue Engineering, Scaffold, Nanocomposite, Bioglass, Osteoblast

Ps-57: Expression of Putative Cancer Stem Cell Marker CD44 in Prostate Carcinomas

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Objective: Cancer stem cells (CSCs) are the main players of prostate tumorigenesis thus; characterization of CSCs can pave the way for understanding the early detection, drug resistance, metastasis and relapse. The current study was conducted to evaluate the expression level and clinical significance of the potential CSC marker CD44 in a series of prostate tissues.
Materials and Methods: One hundred and forty eight prostate tissues composed of prostate cancer (PCa), high-grade prostatic intraepithelial neoplasia (HGPIN), and benign prostate hyperplasia (BPH) were immunostained for the putative CSC marker CD44. Subsequently, the correlation between the expression of these markers and the clinicopathological variables was examined.
Results: Higher level of CD44 expression was observed in 42% of PCa, 57% of HGPIN, and 42% BPH tissues. Statistical analysis showed an inverse significant correlation between CD44 expression with Gleason score of PCa ($P = 0.02$), indicating the higher expression level of CD44 in PCa cases with lower Gleason score.
Conclusion: The higher immunoreactivity of CD44 was observed in almost 50% of prostate samples, which did not in line with the characterization of CSCs as a rare population (<1%) of total PCa cells. Thus, a certain conclusion to evaluate this marker as targets for the progress of new therapeutic strategies in human prostate cancer require further clinical investigations of CD44 as putative CSC markers.
Keywords: Prostate Cancer, Tissue Microarray, Immunohistochemistry, CD44

Ps-58: Differential Expression of Lgr5, a Potential Cancer Stem Cell Marker, in Gastric Cancer Subtypes

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Objective: Cancer stem cell (CSC) based gene expression signatures are associated with tumor initiation, progression, and matastasis in various tumor types. Recently, the characterization and isolation of CSC biomarkers have gained considerable interest. The present investigation was designed to assess the expression levels of Wnt target gene Lgr5 as specific markers of gastrointestinal malignancy and its clinical significance in a series of Gastric Cancers (GC).
Materials and Methods: Lgr5 expression was examined in a well-defined series of 114 gastric tissues, including 81(71%) of intestinal and 33(29%) of diffuse subtypes, which were embedded in tissue microarray (TMA) blocks using immunohistochemistry. The correlation of Lgr5 expression with clinicopathologic parameters was also assessed.
Results: Among 114 gastric cases, higher immunohistochemical expression of Lgr5 was detected in 46% of intestinal and 24% of diffused subtypes. Univariate analysis showed an inverse significant difference between Lgr5 expression with GC subtypes (P=0.02) and tumour differentiation (P= 0.001), indicating the higher level of Lgr5 expression in intestinal subtype (P=0.02) and well differentiation cases (P= 0.001).
Conclusion: Our observation showed that the higher level of Lgr5 expression was not detected in highly aggressive growth form of gastric cancer. These findings warrant further investigation of Lgr5 as a potential CSC marker to understand its value as a target for the development of new therapeutic strategies in human GC.
Keywords: Gastric Cancer, Tissue Microarray, Immunohistochemistry, Lgr5

Ps-59: The Effect of Platelet-Rich Plasma, Bioactive Glass and Hydroxyapatite on Osteogenesis of 3D Human Bone Marrow Derived Mesenchymal Stem Cells

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Objective: Nowadays, mesenchymal stem cells are a valuable source for cell therapy, tissue engineering and regenerative medicine. They have a good potential to differentiate to many cell types such as osteocytes, adipocytes and chondrocytes. Capturing the full potential of MSC will likely require the development of novel *in vitro* culture techniques and devices. Here, we describe a novel and efficient system in order to culture of MSCs as a 3D microaggregates and investigate effects of PRP, bioactive glass and Hydroxiapatite on MSCs osteogenic differentiation.
Materials and Methods: MSCs were cultured into microwells(made from PDMS) and formed hundreds of 3D microaggregates in osteogenic induction medium. Alizarin Red staining, Calcium content assay and ALP activity were performed to evaluation osteogenesis. Results were comprised with 2D culture of MSCs as control group.
Results: Three D microaggregates and all three factors significantly increase calcium content and ALP activity than 2D culture of MSCs. PRP treated microaggreagate had a higher calcium content and ALP activity than others.
Conclusion: It seems that combine of a novel cell culture technique (microaggregates) and active factors can increase osteogenic differentiation than traditional culture system.
Keywords: Mesenchymal stem cells, Osteogenesis, 3D Microaggregate, Platelet-Rich Plasma

Ps-60: The Effect of Bioactive Glass and Hydroxyapatite on BM-derived MSC Osteogenesis in 3D cultures

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Objective: Nowadays, mesenchymal stem cells are a valuable source for cell therapy, tissue engineering and regenerative medicine. They have a good potential to differentiate to many cell types such as osteocytes, adipocytes and chondrocytes. Capturing the full potential of MSC will likely require the development of novel *in vitro* culture techniques and devices. Here, we describe a novel and efficient system to culture MSCs in 3D microaggregates composed of cells and bone mimicking inorganic nano-sized compounds and investigate their effects on MSC osteogenesis.
Materials and Methods: MSCs were cultured in microwells (custom made from PDMS) to form 3D microaggregates in osteogenic induction medium. In order to induce 3D micro bone formation of MSC-inorganic compounds, PDMS microwell inserts were surface treated with 5% pluronic acid to make them non-adherent. Alizarin Red staining, Calcium content assay and ALP activity is to be performed to evaluate osteogenesis. Classic 2D cultures would serve as control group.
Results: Our results showed that by using surface treated PDMS microwells, no cytotoxic effects was detected and that MSC and inorganic bone-mimicry inorganic compounds can form homogenous 3D microaggregates. The resultant composite cell-inorganic compound cultured for two weeks under osteogenic induction medium showed prolonged formidability and rigidity. We would expect higher mechanical strength in composite endowed micro aggregates in comparison with only cell groups
Conclusion: Bone mimicry micro tissues composed of both cells and inorganic compounds (HydroxYapatite and Bioactive glass) can pave the way for macro tissue formation, as one can have enough control over cell type and the inorganic component in each part of the macro tissue. We expect that our micro bone aggregates can be of so much use in the field of bone tissue engineering.
Keywords: Mesenchymal Stem Cells, Osteogenesis, 3D Micro-aggregates, Hydroxyapatite, Bioactive Glass

Ps-61: Osteogenic Potential of Stem Cells-Seeded Bioactive Glass/Gelatin Nanocomposite Scaffolds: A Comparative Study between Human Mesenchymal Stem Cells Derived from Bone, Umbilical Cord Wharton's Jelly and Adipose Tissue

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Objective: In this study, the osteogenic potential of three different mesenchymal stem cells (MSCs) derived from human bone marrow (BM-MSCs), umbilical cord Wharton's jelly (UC-MSCs) and adipose (AD-MSCs) tissues have been examined on nanocomposite scaffolds (bioactive glass/gelatin) implanted in rat critical size calvarial defects.
Materials and Methods: After isolation, culture and characterization, the MSCs were expanded and seeded on the scaffolds for *in vitro* and *in vivo* studies. After conducting the cytotoxicity assay, attachment and growth of the cells on the scaffolds were evaluated. In order to evaluate the scaffolds *in vivo*, adult Wistar male rats were randomly categorized in different groups and treated with the BM-MSCs/scaffolds, UC-MSCs/scaffolds, and AD-MSCs/scaffolds. After 4 and 12 weeks of post-implantation, the animals were sacrificed and the bone healing process was studied.
Results: The results of cytotoxicity assay showed that the scaffolds were highly biocompatible with appropriate cell attachment properties. Moreover, the histological and immunohistological observations showed higher osteogenesis capacity in the group treated with the BM-MSCs/scaffolds compared to other groups. However, the formation of new blood vessels was evidently higher in the defects filled with the UC-MSCs/scaffolds.
Conclusion: This pre-clinical study demonstrates the proof-of-concept that necessitates clinical evaluations.
Keywords: Bone Regeneration, Bioactive Glass-gelatin Nanocomposite, Mesenchymal Stem Cells, Scaffold, *In Vivo* Study

Ps-62: The Effects of 5'-Chloro-5'-deoxy-(±)-ENBA, A Selective A1 Adenosine Receptor Agonist, on Proliferation, Differentiation and Migration of Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cells

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Objective: Proper remyelination is a fundamental process in promoting nervous system regeneration during neurodegenerative disorders, including multiple sclerosis (MS). Insufficient oligodendrocyte progenitor cells (OPCs) migration into demyelinated lesions or inability of these cells to different into mature, functional, myelinating oligodendrocytes are the most prominent causes of remyelination failure. It was reported that adenosine which is a purine nucloside, exerts neuroprotection effects by mainly acting at A1 adenosine receptor (A1AR).
Materials and Methods: Using Bromodeoxyuridine (BrdU), RT-PCR and immunofluorescence assays, we determined the effects of 48 hours treatment with 5'-Chloro-5'-deoxy-(±)-ENBA (5'Cl5'd-(±)-ENBA), a selective and potent A1AR agonist on proliferation, differentiation and migration of human embryonic stem cell (hESC)- derived OPCs in the presence of growth factors.
Results: 5'Cl5'd-(±)-ENBA treatment decreased hESC-derived OPC proliferation significantly. Gene expression analysis of cell cycle regulators revealed a significant up- regulation of p27Kip1 and p21Cip1, which was accompanied with non-significant down-regulation of CYCLIN D1. To clarify to which extend changes in cell cycle regulators gene expression associated with hESC- derived OPC differentiation, the expression level of oligodendrocyte lineage markers, galactocerebrosidase (GALC), proteolipid protein (PLP) and O4 have been detected. The results indicated a significant increase in GALC and non-significant decrease in PLP gene expression levels. However, there was no change in O4 expression level as determined by immunofluorescence staining. There was also a non-significant dose dependent increase in hESC-derived OPC migration after 5'Cl5'd-(±)-ENBA treatment for 24 hours.
Conclusion: It seems A1AR activation with 5'Cl5'd-(±)-ENBA accelerates hESC-derived OPC migration and may predispose these cells for differentiation.
Keywords: A1 Adenosine Receptor, Oligodendrocyte, Proliferation, Differentiation, Migration

Ps-63: Stem Cell Protein, Piwil2, Enhances Stemness and Proliferation of Prostate Cancer LNCaP Cell Line

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Objective: Being the most common malignancy, prostate cancer accounts for the second most cancer –related deaths among men in the western countries. Malignancy is caused by deregulation of normal stem cells which turn them into cancer stem cells (CSCs). Obviously, there must be some genes that their regulation can change the fate of the cells by altering the signaling pathways of the cells. In this research, the effect of Piwil2 -a member of AGO/PIWI gene family- overexpression on the pluripotency and proliferation of prostate cancer cells (LNCaP cell line) was investigated.
Materials and Methods: LNCaP cells were transfected by pcDNA3 plasmid carrying Piwil2 gene under the control of CMV promoter utilizing Neon Electroporation method.

After the selection and establishment of LNCaP-Piwil2 cell line, doubling time test was performed to calculate the proliferation rate of the transfected cells compared to that of control cells. Furthermore, RT and real-time PCR were also carried out for molecular analysis.
Results: The results of doubling time test revealed an eight-hour reduction in the doubling time of the cells from 29 hours to 21hours for LNCaP and LNCaP-Piwil2 cells, respectively. The detection of gene expression by real-time PCR demonstrated a significant increase in the expression of pluripotency factors (Oct4, Sox2, Nanog, c-Myc and Klf4) as well as the genes promoting proliferation or suppressing apoptosis including PCNA, Stat3 and BCL-XL.
Conclusion: As a conclusion according to the data from this study, the overexpression of the above-mentioned genes together with the increase in the proliferation rate in the transfected cells validate that Piwil2 overexpression can indeed promote the pluripotency and proliferation state in LNCaP cells.
Keywords: Piwil2, Prostate Cancer, Proliferation, Pluripotency

Ps-64: Thymoquinone Induces Apoptosis in Glioblastoma Cells via GSH Depletion, ROS Generation, and Mitochondrial Dysfunction

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Objective: Glioblastoma multiforme is among the most lethal types of human cancers and the median survival time of patients is about 14 months. Thymoquinone (C10H12O2), a dietary phytochemical, is the main bioactive component of the volatile oil of the black seed (Nigella sativa, Ranunculaceae family), and has anti-oxidant, anti-inflammatory, and anti-neoplastic properties. Studies have shown the cytotoxic effects of thymoquinone on several types of cancer cell lines. In glioblastoma, thymoquinone was shown to have antiproliferative effect through induction of apoptosis. Present study was carried out to determine the mechanism of thymoquinone induced apoptosis in human glioblastoma cell line (U87MG).
Materials and Methods: U87MG cells were cultured in DMEM/F12 supplemented with 10% FBS at 37 °C and 5% CO2 in a humidified incubator. Cell were treated with temozolomide for 72 h. DNA fragmentation, cytochrome C release, mitochondrial potential, reactive oxygen species generation, glutathione depletion assays and real time RT-PCR were performed for detection of apoptosis pathway. Data were analyzed by one-way ANOVA and $P<0.05$ was considered significant.
Results: Thymoquinone effectively triggers apoptosis in glioblastoma cells. The thymoquinone-induced apoptosis was found to be associated with glutathione depletion, reactive oxygen species generation, mitochondrial transmembrane potential dissipation, upregulation of p53 and Bax, downregulation of Bcl-2, cytochrome C release, activation of caspases (caspase 9 and 3). However, caspase-8 activity did not exhibit significant changes in the thymoquinone-induced apoptosis.
Conclusion: Thymoquinone may become a potential lead compound for future development of anti-glioma therapy.
Keywords: Glioblastoma Multiforme, Thymoquinone, Apoptosis, Caspase Enzyme

Ps-65: Fabrication of Biological Scaffold by HepG2 Cells in 3D Collagen Culture

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Objective: HepG2 cells are being considered as a model for bio artificial liver and liver toxicity studies. According to previous studies, in compare to 2D cultures, HepG2s in 3D immediately display key morphological and functional characteristics of native hepatocytes, including cytochrome P450-mediated metabolism. We hypothesized that the culture of HepG2 cells in 3D culture system can improve hepatocyte differentiation. With regard to these considerations, the current study aimed to fabricate a biological scaffold by culture of HePG2 in 3D collagen scaffold.
Materials and Methods: The human HepG2 cell line (hepatic carcinoma cells) was prepared from the National Cell bank of Iran (NCBI, Pasteur Institute of Iran). HepG2 cells were cultured in 3D collagen scaffold in DMEM, supplemented with 10% (v/v) FBS, 10 µg/mL streptomycin and 100 mg/mL penicillin. The cell were made extracellular matrix and added to collagen during 2 weeks. Afterward decellularization and lyophilization has been done on 3D matrixes. Gycosaminoglycans(GAGs) was identified in extracellular matrix of HepG2 cells by safarin O and alcian blue staining.
Results: It was showed that hepG2 cells were added glycosaminoglycans to collagen. The matrix of the cells become blue after staining with alcian blue and red after straining with safranin O.
Conclusion: The hepG2 cell can be considered as a good candidate cell for making biological scaffold for hepatocyte differentiation.
Keywords: HepG2 Cell, Scaffold, Differentiation, Extracellular Matrix

Ps-66: Direct Conversion of Mouse Embryonic Fibroblasts into Renal Lineage by Establishment of An Initial Epigenetic Activation Phase

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Objective: Considering the increasing prevalence of kidney diseases and the fact that current treatments are ineffective; cell therapy has been introduced as a promising treatment.

Pluripotent stem cells are one of the available cells sources for cell therapy; however, their their use is limited by ethical problems, immunological rejection and the ability to form teratomas in the production of stem cell lines. .In principle, lineage reprogramming, which can be defined as the direct induction of functional cell types from one lineage to another without passing through an intermediate pluripotent stage, could become an alternative to produce the desired cell types. In this study we aim to directly convert primary mouse embryonic fibroblasts into renal lineage cells.

Materials and Methods: We make epigenetic instability in mouse embryonic fibroblasts by transducing Yamanaka factors in reprogramming medium for generating unstable intermediate cells. Afterward we hijack unstable intermediate cells to renal progenitor cells by means of defined nephrogenic medium including small molecules CHIR99021 and TTNPB by activating Wnt, retinoic acid (RA), and bone morphogenetic protein (BMP) pathways.

Results: Mouse embryonic fibroblasts directly were converted into nephron progenitor like cells expressing PAX2, OSR1, WT1 after culturing in nephrogenic medium for 4-5 days. 3 days of transgenic expression of Yamanaka factors in reprograming medium was sufficient for generating of SSEA1+, NANOG- unstable intermediate cells.

Conclusion: Our findings suggest that direct conversion of mouse embryonic fibroblasts into renal lineage cells may be feasible, opening the way for renal repair and regeneration.

Keywords: Direct Conversion, Nephron Progenitor, Small Molecule

Ps-67: Evaluation of Hsv-1 MicroRNAs Effects on Brain Cell Ion Channel Potein Regulation and Their Connection in Epilepsy Specially Epileptic Encephalophaty

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Objective: Primary or idiopathic epilepsies are defined as a heterogeneous group of seizure disorders that show no underlying cause other than a possible inherited predisposition. The genetic basis for two human idiopathic epilepsy syndromes with neonatal and childhood onset has now been pinpointed to specific ion channel proteins for voltagegated potassium or sodium channel subunits. Generalized Primary or adiopathic epilepsies are defined as a heterogeneous group of seizure disorders that show no underlying cause other than a possible inherited predisposition. The genetic basis for two human idiopathic epilepsy syndromes with neonatal and childhood onset has now been pinpointed to specific ion channel proteins for voltagegated potassium or sodium channel subunits. So some of studing shows that many of mutation ione channel interfere in epilepsy such as sodium ,pottasuim,calcium channel and solute carriers.

Materials and Methods: We have decided to study that which genes are involve in epilepsy with newe view.findings show that mutation or structural defection in some ion channel can cause of epilepsy.we find this ione channel such as SCN8A and slc22A2 that mir-H2 can target these. So,we extract mir-H2 plasmid and culture in HEK cell line. extract RNA and build cDNA.we chek their expression with real time PCR.and show increase in expression .but now we just design specific primer that see connection between this channel and mir-H2.

Results: The most cause of epilepsy are mutation in ione channels and solute carriers such as:sodium, pottasuim ,calcium channels. We want to use of HSV1'miRNA's to see that they can target this channel or not. Because both location of them are brain, the functional of mir-H is the same as other human miRNA and we want to know they can affect on ione channel or not,and how they can affect on them?

Conclusion: Epilepsy is one of the most common neurological affliction.previous epidemiological analyses have shown that nervous system (CNS) infection is a major cause of acquired epilepsy. Viral encephalopathies is a relatively significant risk factor for developing unprovoked seizures in patients with previous CNS infection. HSV1 is one such virus that has been linked with epileptic seizure, an idea first described with Ross in 1972. It is a neuropathic virus and can travel to the neuronal cell body in the brain stem, cereberum and specially temporal rejon from the inoculation site bye retrograde axonal transport. Primery HSV1 infection,which are most often insidious and asymptomatic,usually occur in young children. Using polymerase chain reaction (PCR), HSV genomic sequences could be detected in one-third normal human brains,suggesting that the virus establishes latency in the brain.typical HSV encephalit mostly resulting the spread of reactivated latent virus. Clinical manifestation of typical HSVE is a rapidly progressive fatal course usually accompanied by seizure (37-68%), and most survivors have epilepsy and neurological morbidity. The HSV-positive rate in surgical specimens from patient with intractable epilepsy is significantly higher than in those with epilepsy. These finding imply a strong correlation between HSV1 infection and epileptic seizure.

Keywords: MiRNA, Herpes Simplex One, Epilepsy, Ione Channel, Seizure

Ps-68: Human Umbilical Venous Endothelial Cells-Conditioned Medium Improve The Acute Renal Failure Induced by Cisplatin in Male Wistar Rat

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Objective: Acute renal failure (ARF) is the generic term for an abrupt and sustained decrease in renal function . Human umbilical venous endothelial cells (HUVECs) secrete many growth factors and cytokines that to be beneficial for the renal recovery after ARF. In this study, we used of HUVEC-conditioned medium (HUVEC-CM) that contain of various factors to improve ARF induced by cisplatin.

Materials and Methods: Model of ARF induced by in-

traperitoneal injection of 5mg/kg cisplatin in wistar rat. In experimental group, HUVEC-CM was injected through intraperitoneal injection in cisplatin-induced ARF rat model. Vehicle group was exposed with cisplatin and normal saline. The animals were followed for biochemistry analysis and pathology.
Results: HUVEC-CM was administered to rat 3h after injection of cisplatin, and the therapeutic effect was evaluated after 5 days from cisplatin injection. Experimental groups that received 1 mg/kg of HUVEC-CM demonstrated significantly lower serum creatinine and urea after 5 days compared with models and vehicle groups. Also, necrosis and hyaline casts were decreased in experimental groups compared with cisplatin-induced rats in the model and vehicle groups.
Conclusion: These data demonstrate that HUVEC-CM certainly protect tubular and glomerular injury against renal failure.
Keywords: Acute Renal Failure, Human Umbilical Venous Endothelial Cells, Conditioned Medium, Cisplatin

Ps-69: L-Carnitine Could Increase Human Telomerase Reverse Transcriptase Gene Expression and Telomere Length of The Human Adipose-Derived Mesenchymal Stem Cells Obtained from The Aged Subjects

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Objective: The amino acid derivative, L-carnitine takes part in many functions in organism, such as lipid catabolism and energy production with antiapoptotic properties. It is believed that L-carnitine as an antioxidant plays an important role in scavenging reactive oxygen species and protecting against accelerated aging of the skin and muscles. Human mesenchymal stem cells are also attractive candidates for cell therapy and regenerative medicine due to their multipotency and ready availability, but their application can be complicated by the factors such as age of the donors and senescence-associated growth arrest during culture conditions. The aim of this study was to evaluate of the effect of L-carnitine on the human telomerase reverse transcriptase, hTERT, gene expression and telomere length in aged human adipose-derived mesenchymal stem cells.
Materials and Methods: For this purpose, cells were isolated from healthy aged volunteers and their viabilities were assessed by MTT assay. Quantitative gene expression of hTERT and absolute telomere length measurement were also performed by real-time PCR in the absence and presence of different doses of L-carnitine (0.1, 0.2 and 0.4 mM).
Results: The results indicated that L-carnitine could significantly increase the hTERT gene expression and telomere length, especially in dose of 0.2 mM of L-carnitine and in 48 h treatment for the aged human adipose-derived mesenchymal stem cells samples.
Conclusion: It seems that L-carnitine would be a good candidate to improve the lifespan of the aged adipose -derived mesenchymal stem cells due to over-expression of telomerase and lengthening of the telomeres.
Keywords: L-carnitine, Stem Cells, Gene Expression, Telomerase

Ps-70: Correction of Chromosomal Abnormalities following Treatment of Mosaic Mouse Embryonic Stem Cells with Lapatinib and Paclitaxel

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Objective: One of the main reasons for failure of assisted reproductive technology (ART) especially *In vitro* fertilization (IVF) is high incidence of chromosomal abnormalities in embryos that mostly are diploid-aneuploid mosaic. mESC-based systems can be used as a model to investigate the trend of preimplantation human embryo development and used for embryo toxicity screening. Recent studies show more sensitivity of aneuploid cell lines to some anticancer drugs compared with normal cells. In this study, we attempted to evaluate such effects of two anticancer drugs, lapatinib and paclitaxel in mESCs. Lapatinib is a small molecule that plays a survivin antagonist role whilst survivin is overexpressed in cancer cells. Paclitaxel as a microtubule stabilizer targets cell cycle.
Materials and Methods: To assess cell toxicity of lapatinib and paclitaxel, B5 mosaic mESC line was exposed to different concentration of these drugs according to previously reported IC50 and EC50.Treatment with both of drugs was performed in two groups, first with once treatment , and the second with treatment in five continues passeges.To investigate the cytogenetical effects of lapatinib and paclitaxel, treatment and control groups were studied using G-band karyotyping. The stemness characterization of mESCs cells treated with paclitaxel and lapatinib was demonstrated by alkaline phosphase staining.
Results: According to doubling time of mESCs 0.01µM for 8 hours and 0.2 µM for 24 hours were selected for paclitaxel and lapatinib treatment, respectively. In the second group of treatments and control, the rate of numerical abnormalities after five passeges were higher than first passage while the first group of treatments had normal karyotype. We confirmed that treatment of mESCs with paclitaxel and lapatinib pluripotency according alkalin phosphatase staining finding.
Conclusion: Alternative use of lapatinib and paclitaxel in culture medium in some passeges seems to increase chromosomal stability. More evaluation of the probably effects of drugs treatment on pluripotency is under investigation.
Keywords: Lapatinib, Paclitaxel, mESCs, Karyotype, Aneuploidy

Ps-71: Using Herbal Preconditioning, Extract of Origanum Vulgare Protects The Mesenchymal Stem Cells from The Oxidative Stress

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Objective: One of the most important challenges in mesenchymal stem cells (MSCs) mediated cell therapy is their low survival rate following transplantation. Therefore, a great number of preconditioning studies have been conducted to potentiate the MSCs against stresses. This study was aimed at preconditioning of the MSCs by using the high concentrations of Origanum Vulgare extract to protect them against oxidative stress.

Materials and Methods: MSCs were isolated from the bone marrow of rat. Then, they were preconditioned with the high doses (80-0.625 mg/ml) of Origanum Vulgare extract for one hour. Following two days recovery period, MSCs were exposed to the half maximal inhibitory concentration (IC50) of hydrogen peroxide as an oxidative stress agent for four hours. After two days recovery again, MSCs survival and apoptosis were evaluated by using MTT and caspase 3 assays, respectively.

Results: Preconditioning of the MSCs with Origanum Vulgare extract at2.5mg/ml significantly increased the MSCs survival from 46% in H2O2-induced MSCs to 74% preconditioned-MSCs and significantly decreased their caspase 3 from 229% in H2O2-induced cells to 116% when they were preconditioned with Origanum Vulgare extract ($P<0.05$).

Conclusion: Herbal preconditioning with the higher doses of Origanum Vulgare can protect mesenchymal stem cells against oxidative stress and this strategy may be suggested to prevent the cell death in transplantation programs.

Keywords: Preconditioning, Herbal Extract, Origanum Vulgare, Mesenchymal Stromal Cells

Ps-72: Overexpression of MicroRNA-148b-3p Stimulates Osteogenesis of Human Bone Marrow-Derived Mesenchymal Stem Cells

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Objective: Bone formation process is controlled by many regulatory factors including microRNAs. MicroRNAs (miRNAs) are small non-coding endogenous RNAs which play crucial role in regulating gene expression in many aspects of bone development and metabolism. Beside the role of miRNA-148b in osteogenesis, miRNAs could be promising therapeutic agents in bone tissue formation. To evaluate the effects of miR-148b-3p on osteogenic activity, we introduced lentiviral-miR-148b-3p-expression vectors into human mesenchymal stem cells (hMSCs).

Materials and Methods: To do so, the sequence of hsa-miR-148b-3p was inserted to lentiviral vector using cloning procedure. The successful introduction of the desired sequence was confirmed by analytical digestion and nucleotide sequence analysis. After that, lentiviral-miR-148b-3p vectors were constructed by multiple plasmid transient transfection protocol using human embryonic kidney (HEK) 293T cells. Then, MSCs, isolated from bone marrow and characterized, were infected with current lentiviruses and osteoinducted for a frame of 21 days. Finally, treated cells were subjected to alizarin red S (ARS) as well as alkaline phosphatase (ALP) staining and activity, and real-time polymerase chain reaction for osteoblast gene markers.

Results: Transduction efficiency of BM-MSCs was more than 90% three days post-transduction, which revealed our culture model successfully overexpressed miR-148b-3p. The delivery of miR-148b-3p raised osteogenic differentiation of BM-MSCs as it was shown by ARS and ALP staining and activity. In support of these data, overexpression of this microRNA dramatically up-regulated the expression of osteoblast differentiation markers.

Conclusion: Since lentivirally overexpression of miR-148b-3p could drive differentiation of osteoblasts, this agent could be applied in the future as a therapeutic biomarker in RNA-based drugs for optimal bone function.

Keywords: Stem Cells, MicroRNA-148b, Cloning, Lentiviral Vectors, Osteogenesis

Ps-73: Induction of Neural Phenotype in Stem Cells by Neonatal Rat Brain Extract

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Objective: Parkinson disease is the second most common neurologic disorder in the World that destroys dopaminergic neurons in substantia nigra pars compacta. As treatments for Parkinson disease, many therapeutic procedures have been used. Cell therapy is one of the novel methods using stem cells. P19 embryonic carcinoma cells are a type of stem cell lines having a high proliferation rate in the culture medium. As P19 cells are exposed to various inducers they may differentiate into derivative cells of the three embryonic germinal layer.

Materials and Methods: P19 cells were exposed to neonatal rat brain extract to differentiate into neural phenotype. α-MEM medium supplemented by 10% bovine serum was used. A concentration of 100 ng/ml neonatal rat brain extract was used to differentiate P19 cells into neural phenotype. Cresyl violet staining method was used to investigate the morphology of differentiated cells. The expression of the specific neural gene like tyrosine hydroxylase was determined by Real-time PCR method.

Results: Morphological investigation of the differentiated cells using Cresyl violet staining exhibited the induction of

neural phenotype in P19 cells. In addition, the expression of specific neural genes like tyrosine hydroxylase in P19 cells was confirmed.
Conclusion: The results of this study indicated that embryonic carcinoma stem cells can differentiate into neural as they were exposed to neonatal rat brain extract.
Keywords: Embryonic Carcinoma Cells, P19 Cell Line, Brain Extract, Neuronal Differentiation, Tyrosine Hydroxylase Gene

Ps-74: Liver Regeneration in A Carbon Tetrachloride–Induced Acute Liver Failure Model: Do Bone Marrow-Derived Cells Contribute?

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Objective: Here a liver failure model is introduced by carbon tetrachloride (CCL4) to assess whether bone marrow-derived progeny contribute to liver regeneration after acute hepatotoxic liver failure. Adult bone marrow contains progenitors capable of generating hepatocytes.
Materials and Methods: Control group was performed by injecting only saline in CCL4-treated animals. In bone marrow mononuclear cells (BMMCs) treated group (group 1), BMMCs were obtained from human bone marrow (BM) injected into the rats 24 h after liver damage with CCL4. Also in hepatocyte-like cells-treated group (group 2), the animals were infused with BMMCs derived hepatocyte-like cells 24 hours after CCL4 injection. Transaminases and survival rate were measured at 72 hours, 1, 2, 3, 4 weeks after injection of either BMMCs or hepatocyte-like cells. Also, BMMCs in blood of rats were detected by flowcytometry with monoclonal antibody specific for CD 45, CD 34 and CD 14 after 4 weeks. Liver histology and immunohistochemistry were observed 2w and 4w after injury.
Results: Homing of BMMCs was detected in the peripheral blood of the treated rats by flowcytometric analysis with monoclonal antibodies specific for CD 45, CD34 and CD14. Serum Alanine transferase (ALT) and Asprtatetransferase (AST) were significantly reduced in the treated groups after 72 h. Liver histopathological examination revealed significant decrease in the degree of necrosis, steatosis and inflammation with significant increase in the mitotic index of both treated groups than non-treated group, indicating that liver was already in its regenerative process. Liver immunohistochemical stained section from both treated groups showed significant positivity for both anti-human albumin and proliferating cell nuclear antigen (PCNA) indicating the presence of functional human cells in rat livers and proliferation of liver cells.
Conclusion: BMMCs or hepatocyte-like cells injected IV via the tail vein in an animal model of acute liver failure can engraft the liver and are able to reduce liver scarring as well as stimulating regenerative process. So results indicate that BMMCs transplantation have potential as a new therapeutic option for acute liver disease and suggest that these cells may contribute to hepatic recovery.
Keywords: Acute Liver Failure, BMMCs, Hepatocyte-like Cells, Carbon Tetrachloride, Animal Model

Ps-75: Nanotechnology to Drive Stem Cell Commitment in Liver Tissue Engineering

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Objective: The objective of this review was to set a baseline for the current work being conducted in developing of advanced techniques to understand and control functions of micro environmental signals and novel methods to track and guide transplanted stem cells. Tissue engineering and regenerative medicine have been constantly developing of late due to the major progress in cell and organ transplantation, as well as advances in materials science and engineering. Although stem cells hold great potential for the treatment of many injuries and degenerative diseases, several obstacles must be overcome before their therapeutic application can be realized.
Materials and Methods: The review was implemented by presenting a current and emergent approach based on stem cells in the field of liver tissue engineering for specific application.
Results: The combinations of stem cell therapy and nanotechnology in tissue engineering and regenerative medicine allow nanotechnology to engineer scaffolds with various features to control stem cell fate decisions. Fabrication of Nano fiber cell scaffolds onto which stem cells can adhere and spread, forming a niche-like microenvironment which can guide stem cells to proceed to heal damaged tissues.
Conclusion: The combination of stem cells and tissue engineering opens new perspectives in tissue regeneration for stem cell therapy because of the potential to control stem cell behavior with the physical and chemical characteristics of the engineered scaffold environment. The application of nanotechnology to stem cell biology would be able to address the challenges of a major complication encountered with stem cell therapies, the failure of injected cells to engraft to target tissues.
Keywords: Nanotechnology, Stem Cell, Regenerative Medicine, Tissue Engineering, Liver

Ps-76: Wound Healing and Skin Rejuvenation by Shed Microvesicles from Embryonic Stem Cells under Aloe Vera Barbadenisis Induction

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Objective: In addition to differentiation into many cell types, stem cells are applied widely in therapeutic strategies. In this regard, stem cell therapy in wound healing and skin rejuvenation is expanding. Microvesicles are extracellular liposomes which are shed from many cell types. They contain mRNAs, non-coding RNAs and proteins, so, microvesicles could transfer signals among the cells. This characteristic makes them appropriate for therapeutic approaches. Aloe Vera, as a tropical plant, is utilized in skin burns and wound healing. Mucilage of the leaves of this plant contains glycoprotein that speeds up the wound healing process. Also, its polysaccharide drives the cell growth and skin repair.
Materials and Methods: In this study, with the aim to investigate the effect of shed microvesicles from ESCs on the restoration and Rejuvenation, first the cells were induced by Aloe Vera. Then shed microvesicle in both test and control groups were isolated by ultra-centrifugation. SEM and DLS analysis confirmed the microvesicle isolation. Whole content of RNA of microvesicles was extracted and cDNA was synthesized. In the next step, primers were designed for real-time PCR of PDGF, OPN, FGF, CCN1, AQP3 and HSP70. Investigation of transcriptional alteration of these genes could help us to understand the role of Aloe Vera in upregulation of wound healing and skin rejuvenation markers.
Results: In this study, with the aim to investigate the effect of shed microvesicles from ESCs on the restoration and Rejuvenation, first the cells were induced by Aloe Vera. Then shed microvesicle in both test and control groups were isolated by ultra-centrifugation. SEM and DLS analysis confirmed the microvesicle isolation. Whole content of RNA of microvesicles was extracted and cDNA was synthesized. In the next step, primers were designed for real-time PCR of PDGF, OPN, FGF, CCN1, AQP3 and HSP70. Investigation of transcriptional alteration of these genes could help us to understand the role of Aloe Vera in upregulation of wound healing and skin rejuvenation markers.
Conclusion: In this study, with the aim to investigate the effect of shed microvesicles from ESCs on the restoration and Rejuvenation, first the cells were induced by Aloe Vera. Then shed microvesicle in both test and control groups were isolated by ultra-centrifugation. SEM and DLS analysis confirmed the microvesicle isolation. Whole content of RNA of microvesicles was extracted and cDNA was synthesized. In the next step, primers were designed for real-time PCR of PDGF, OPN, FGF, CCN1, AQP3 and HSP70. Investigation of transcriptional alteration of these genes could help us to understand the role of Aloe Vera in upregulation of wound healing and skin rejuvenation markers.
Keywords: Microvesicles, Stem Cell, Aloe Ver, Wound Healing

Ps-77: All Trans Retinoic Acid Preconditioning to Improve Angiogenic Mechanisms of Mesenchymal Stem Cell

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Objective: Impaired neovascularization of the skin, nerves and myocardium lead to the major appearance of diabetes, including impaired wound healing, neuropathy and poor prognosis after myocardial infarction. Recently, stem cell based therapies is considered a promising alternative in treatment of various diseases. Stem cell preconditioning with chemical and pharmacological agents has been indicated to enhance therapeutic efficacy. Therefore, in the current study, we sought to explore the effect of all trans retinoic acid (ATRA) on some mediators involved in survival and angiogenesis of mesenchymal stem cell (MSCs) *in vitro* and *in vivo*.
Materials and Methods: MSCs were treated with ATRA and compared to nontreated MSCs for expression of COX2, HIF-1α, VEGF, Ang2 and Ang4 at mRNA levels using qRT-PCR. Prostaglandin E2 (PGE2) production was determined by ELISA assay. The activity of caspase-3 was measured using an enzymatic assay. Angiogenic potential of ATRA was determined by *in vitro* (in HUVECs) and *in vivo* experiments including three -dimensional tube formation testing and surgical wound healing models.
Results: In ATRA- treated MSCs, expression of COX2, HIF-1, VEGF, Ang2 and Ang4 were significantly increased compared to control groups. Elevation of noted genes was reversed by Celecoxib, a COX2 inhibitor. PGE2 generation and MSCs viability were significantly enhanced in contrast to caspase 3 activity. The *in vitro* three-dimensional tube formation as well as *in vivo* wound healing model were also significantly higher than control groups.
Conclusion: ATRA preconditioning improved neovascularization potential of MSCs by induction of COX2/PGE2 and affecting the HIF-1a pathway.
Keywords: Stem Cell Therapy, Prostaglandin E2, Cyclooxygenase-2, Mesenchymal Stem Cells, All Trans Retinoic Acid

Ps-78: Core-Shell Fibrous Scaffold for Retinal Differentiation of Mesenchymal Stem Cells

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Objective: This paper describes the technique of using coaxial electrospinning to generate bicomponent core-shell nanofibers from two fluids poly caprolactone (PCL) and poly ethylene glycol (PEG) for differentiation of conjunctiva mesenchymal stem cells (CJMSCs) into photoreceptor-like cells by delivery of taurine.
Materials and Methods: In the present study, the effect of many parameters such as polymer concentration, nozzle collector distance, applied voltage and outer solution flow rate on creation of the core-shell structure were examined. The morphology and structure of core shell fibers structure was characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and fourier transform infrared spectroscopy (FTIR) and then retinal differentiation of cultivated cells examined by quantitative real time

PCR (qPCR).
Results: SEM images identified significant variation between 25 and 35% PEG concentration groups for fiber diameter. As faster flowing rates from the outer nozzle (PCL fluid) were applied, the possibility creation of fibrous scaffold was increased. The lower diameter and the best quality alignment of core-shell fibrous scaffold were achieved in 22Kv and 24 Kv. As rising distancing were applied, the fibrous diameter increased and the spraying was observed. qPCR analysis demonstrated the expression of rhodopsin (Photoreceptor main marker) of CJMSCs to on PEG/PCL scaffolds included taurine and suggested their potential application in retinal regeneration
Conclusion: According to result, we have proved successful in the creation of core/shell fibrous scaffold of PEG/PCL by ideal processing parameters of coaxial electrospinning for retinal tissue engineering.
Keywords: Coaxial Electrospinning, Core-shell, PEG/PCL, Photoreceptor-like Cells

Ps-79: Induced Pluripotent Stem Cell Differentiation to Oligodendrocyte by miR-219 Overexpression

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Objective: induced pluripotent stem (iPS) cell as a type of stem cell which develops from a patient's own cells, have been produced recently by introducing several different genes into somatic cells. These cells can be derived from any somatic cell and they possess ES cell like pluripotency and proliferative capacity without problems of embryonic or adult stem cell. Then, It could be considered as a good cell source for cell therapy. MicroRNAs are single strand RNAs which have key roles in oligodendrocyte cellular process. Based on recent findings, MicroRNA 219 (miR-219) suppresses negative regulators of oligodendrocyte development and advances oligodendrocyte differentiation. In our study, we differentiated iPS cells to oligodendrocyte by miR-219 transfection.
Materials and Methods: iPS cells were treated for 12 days with fibroblast growth factor 2/epidermal growth factor (20ng/mL) and platelet-derived growth factor (PDGF)-AA (10ng/mL), then, were infected by miR-219. Next, the expressions of stage-specific oligodendrocyte cells markers Nestin, Olig2, Sox10, PDGFRa, CNP, A2B5, O4, and MBP were evaluated by immunocytochemistry and quantitative reverse transcription PCR.
Results: Our analyses represented that the expression of pre-oligodendrocyte markers were increased in the cells infected by miR-219.
Conclusion: The Overexpression of miR-219 is a novel efficient way to differentiate iPS cells to oligodendrocyte. Therefore, iPS cells could be used as a safe cell source for patient specific cell therapy In which cells function in their natural environment, without eliciting chronic immune or inflammatory reactions, and without the problems that would result from the use of immunosuppressive drugs.
Keywords: iPS, miR-219, Oligodendrocyte

Ps-80: Fndc5 Knockdown Did not Alter The Expression of Mesodermal Markers during The Early Stage of Cardiac Differentiation of Mouse Embryonic Stem Cells

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Objective: Fibronectin type I domain-containing 5 protein (Fndc5) is a glycosylated type I membrane protein which is highly expressed in heart, brain and muscle in adult mouse. Previous studies have shown that the transcript levels of Fndc5 gradually increase when mouse embryonic stem cells (mESCs) differentiate into beating bodies. Formation of mesoderm is the first step of cardiomyocyte generation from pluripotent stem cells which is characterized with enhanced expression of mesodermal markers, Brachyury and FoxC1. In order to clarify the role of Fndc5 in the formation of mesodermal cells, this study was performed.
Materials and Methods: We generated a stably inducible transduced mESC line that derives the expression of a short hairpin RNA (shRNA) against FNDC5 gene following doxycycline (Dox) induction. Dox was added during hanging drop formation and the relative expression of mesodermal markers, Brachyury and FoxC1 were assessed on day 4 from the beginning of the experiment during embryoid bodies (EBs) formation.
Results: Real-time PCR data indicated that knockdown of Fndc5 in early stage of cardiac differentiation was not influenced transcript levels of mesodermal markers. While in parallel, transcription levels of stemness markers (Nanog, Oct4) significantly decreased in transduced cells compared to the other groups.
Conclusion: FNDC5 may not exert a functional role in the early stage of cardiac differentiation dissimilar with its previously recognized function in early stage of neural differentiation. Thus FNDC5 may have distinct roles in different cell types.
Keywords: Fndc5, Mesodermal Markers, Mouse Embryonic Stem Cells

Ps-81: Generating An iPS Cell Model of Parkinson's Disease Based on IFNB-Knockout Mice

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Objective: Parkinson's disease is an elusive condition causing the loss of dopaminergic neurons in the Substantia Nigra with not much being known about its underlying mechanisms. Despite many efforts to model this disease both *in vivo* and *in vitro*, until recently none have been completely successful. We are going to use cells from a novel mouse model based on the knockout of Interferon beta (IFNB) to create induced pluripotent stem cells (iPSCs) which will then be differentiated to dopaminergic neurons. Characterizing their Parkinsonian phenotype will result in an established *in vitro* model for Parkinson's disease with many research applications including studies on the underlying mechanisms of this phenotype and it's spreading between neurons and of course drug screening.
Materials and Methods: iPSCs were generated from both KO and WT mouse-fibroblasts using retroviral induction of the four Yamanaka factors Oct4, Sox2, KLF4 and C-Myc. The generated iPSCs were characterized using Alkaline Phosphatase assay as well as immunofluorescence and qRT-PCR to confirm the expression for pluripotency factors. After characterization these cells were differentiated to dopaminergic neurons using the dual SMAD inhibition method of neural induction. Complete differentiation to functional dopaminergic neurons were assessed using immunofluorescence and electrophysiological tests (patch clamp).
Results: Our preliminary results show the aggregation of phosphorylated alpha-synuclein which is the hallmark of Parkinson's disease in the the KO cells, as detected using immunofluorescence, but not in the neurons differentiated from WT iPSCs.
Conclusion: It appears that our iPSC model can be the first in genetic *in vitro* model to show the hallmarks of Parkinson's disease without the need for external stress factors.
Keywords: Parkinson's Disease, iPS Model, Disease Modeling, Interferon Beta

Ps-82: Recombinant Fibroin Containing The RGD Motif Enhances Limbal Mesenchymal Stromal Cell Adhesion and Proliferation

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Objective: At the periphery of the cornea, in a region called the limbus, exists a population of stromal progenitor cells termed limbal mesenchymal stromal cells (L-MSC). It has been proposed that L-MSCs improve wound healing in the cornea by enhancing epithelial cell growth and repair of the stromal tissue. As such, there is widespread interest in exploiting the properties of L-MSCs for the treatment of corneal disease. Thus, studies have been initiated in our laboratory to optimise techniques for the isolation and cultivation of L-MSC. In parallel studies we have demonstrated that silk fibroin (SF) has potential as a scaffold for supporting the growth and implantation of L-MSC. In the current study we report an attempt to optimise L-MSC culture on recombinant silk fibroin containing the RGD motif (RGD-SF), a recognized ligand for cell attachment. Our hypothesis is that L-MSC display enhanced attachment and growth on RGF-SF compared to SF thus providing a superior scaffold for clinical use.
Materials and Methods: Human L-MSC cultures derived from four donors (n=4) were seeded onto either tissue culture plastic, RGD-recombinant fibroin or conventional fibroin for 90 minutes, 6 and 10 days. Differences in L-MSC adhesion and growth were determined photographically and quantified using the PicoGreen assay (measures dsDNA).
Results: At all time points examined, L-MSCs displayed evidence of superior attachment and proliferation on RGD-SF compared with SF as indicated by both morphological examination and PicoGreen assay. Furthermore, morphological examination revealed that while L-MSCs cultured on conventional fibroin generally formed sparsely scattered clusters, those cultured on RGD-SF were more uniform in adherence to RGD-F.
Conclusion: The results suggest that RGD-SF offers superior performance as a scaffold material for L-MSC cultures compared to conventional fibroin. Based on such data we suggest that RGD-SF has potential as a biomaterial for improving strategies for the treatment of corneal disease.
Keywords: Silk fibroin, RGD, Cornea, Limbus, Mesenchymal Stromal Cells

Ps-83: Design and Synthesis of Electroactive Scaffold as a Cardiac Patch

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Objective: Our aim was to fabricate an electroactive composite of collagen , the main extracellular matrix protein, and an electro active component graphene oxide (GO) and reduced graphene oxide (rGO) as a cardiac patch.
Materials and Methods: The 3D collagen (Col) scaffold were fabricated by freeze-drying method and then coated by immersion in GO solution; some of the scaffolds were then reduced by sodium hydrosulfite. The scaffolds were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD) analyses and Thermo gravimetric analysis (TGA). Young modulus and tensile strength of the samples were also assessed by tensile test. Two point probe test was used to measure scaffold's conductivity. Biological properties of each scaffolds were evaluated by contact angle test, scanning electron microscopy (SEM), atomic force microscopy(AFM) and MTT assay.
Results: Our results indicated that the GO-Col scaffolds were comprised of randomly-oriented interconnected pores with average pore size of 115.8 ± 94 μm. Compositional analysis showed that GO was dispersed uniformly throughout the GO-Col matrices. TGA profiles demonstrated that GO-Col scaffolds were thermally stable in spite of adding GO. The tensile

strength was increased by coating different GO concentrations to 173.64Kpa at concentration of 90µg/ml. None of our samples showed toxic effect by MTT assay.Electrical conductivity measured after reducing our samples were in the range of semi-conductive materials which is appropriate for cardiac tissue engineering applications.
Conclusion: It was proved ,in our study, that GO-Col scaffolds have potential as conductive scaffolds for cardiac patch application, although, further investigations are needed to find out several aspects of reaching to a beating cardiac tissue on this kind of scaffold.
Keywords: Cardiac Patch, Conductive Scaffold, Graphene Oxide, Reduced Graphene Oxide, Collagen

Ps-84: Spermatogonial Stem Cells of Azoospermic Patients Can Be Enter into Meiosis *In Vitro*

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Objective: Azoospermia is a condition that causes infertility in men. Due to low efficiency of previous treatments for this disease, today stem cell field are considered as a new therapeutic approach for the treatment of male infertility. Stem cells are undifferentiated cells and are found in different tissues. These cells have capacity of self-renewal and differentiation into other lineages that termed potency. Classification of their potency is: totipotent, pluripotent, multipotent, and unipotent. Spermatogonial stem cells (SSCs) are the multipotent stem cells that considered to use as appropriate source in treatment of azoospermic patients for differentiation into sperm cells.
Materials and Methods: SSCs after mechanical and enzymatic isolation from azoospermic patient's testes biopsies were cultured in T25 flasks. After confluent phase and second passage, the expression of mesenchymal stem cell markers assessed by flowcytometry. In next step SSCs induced by sheep testes extraction for differentiation into sperm cells. Expression of Dazl gene as meiosis marker was investigated by western blotting technique.
Results: After primary culture of SSCs in passage 2 (about 10 days) stem cell markers expression studied by flowcytometry. Flowcytometry results showed that human SSCs highly expressed CD90, CD105 and CD44 and are positive for Stro-1, CD146, CD106 and CD166. Furthermore, the expression of CD19 and CD45 observed in low percentage of these cells. After differentiation these cells showed sperm like shape. Western blotting analysis showed that Dazl proteins have expressed in differentiated SSCs and this cells have entered into the meiosis stage.
Conclusion: SSCs have mesenchymal stem cell markers that can be differentiated into sperm like cells.
Keywords: Western Blotting, Dazl, Sperm, Stem Cells, Spermatogonial Stem Cells

Ps-85: Defined Co-Culture System of Equine Mesenchymal Stem Cells with Neutrophils

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Objective: It has been suggested that mesenchymal stem cells (MSCs) have immunomodulatory effects through interaction with immune system cells. We aimed to define a suitable condition for co-culture of equine adipose-derived MSCs and neutrophils.
Materials and Methods: Frozen equine adipose-derived MSCs (AMSCs) were thawed and their purity and characteristics were confirmed at passage 5. Equine Polymorphonuclear neutrophils (PMN) were isolated by density and hypotonic lysis. Cells were resuspended and neutrophil suspensions were perfused over adherent AMSC (at AMSC to neutrophil ratios of 1:50) in direct contact for 24 h in 12-well plates. After that, probability of pure isolation of AMSCs and neutrophils, and cell viability were checked.
Results: AMSCs showed typical characteristics of MSC such as marker expression (CD29, CD90 and CD44) as well as trilineage differentiation potentials. Neutrophils were not affected by isolation procedure and their normal morphology was confirmed under light microscope. AMSCs strictly adhere to the plates whereas neutrophils were in suspended status during 24 h of co-culture. At the end, supernatants on plate wells containing neutrophils were removed and purified neutrophils were isolated. In next step, pure and alive adherent AMSC was collected using trypsin.
Conclusion: We conclude that MSC can co-culture easily with neutrophils and this culture system can be used for studying interactions between MSc and Neutrophils *in vitro*.
Keywords: Mesenchymal Stem Cell, Neutrophils, Co-culture, Equine

Ps-86: Temozolomide Induces Apoptosis Through Mitochondrial Pathway in U87MG Cell Line

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Objective: Apoptosis is a gene-directed cell death program, occurring without the production of inflammation. The dysfunction of the apoptotic signaling process is often a major causative factor in tumorigenesis, and also renders the cancer cell resistant to treatment. Induction of apoptosis in cells can be an appropriate strategy by which chemotherapeutic agents kill tumor cells. Temozolomide is a novel oral alkylating agent that has been widely used in the treatment of glioblastoma. The aim of the present study was to investigate the mechanism of temozolomide induced apoptosis in human glioblastoma multiforme cell line (U87MG).
Materials and Methods: U87MG cells were cultured in

DMEM/F12 supplemented with 10% FBS at 37 °C and 5% CO2 in a humidified incubator. Cell were treated with 20 μM temozolomide for 72 hours. DNA fragmentation, caspases activity, cytochrome C release, mitochondrial potential assays and real time RT-PCR were performed for detection of apoptosis pathway. Data were analyzed by one-way ANOVA and P<0.05 was considered significant.
Results: Temozolomide-induced U87MG apoptosis was confirmed by our results. Temozolomide treatment increased P53 and Bax mRNA expression levels, reduced Bcl-2 mRNA expression level and triggered the change of mitochondrial membrane potential and the release of cytochrome C from mitochondria into cytosol, which in turn activated caspase-9 and caspase-3, and resulting in apoptosis. However, caspase-8 activity did not exhibit significant changes in the temozolomide-induced apoptosis.
Conclusion: Temozolomide could induce U87MG apoptosis through the mitochondrial-dependent pathway.
Keywords: Glioblastoma Multiforme, Temozolomide, Apoptosis, Caspase Enzyme

Ps-87: Electrospun Nanofibrous Scaffold Based on TPU/CNT Nanocomposite Used in Tissue Engineering Application

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Objective: Tissue-engineering scaffolds play important roles in cell growth and tissue formation. The scaffolds should be analogous to extracellular matrix (ECM) in terms of both chemical and physical features. Hence in the present work, it has been attempted to fabricate electrospun nanofibers based on a flexible thermoplastic urethane (TPU) and carbon nanotubes, for usage as the scaffold for soft tissue regeneration. Thermoplastic polyurethanes (TPU) are elastomeric materials with suitable resistance to microorganisms and abrasion, and also excellent hydrolytic stability. Carbon nanotubes provide nanostructured topography, mechanical strength and flexibility as well as a high aspect ratio in nanoscale and binding capacity to biomolecules.
Materials and Methods: In order to prepare the electrospinning solution, TPU granules were dissolved in THF/DMF (70:30) at a concentration of 6% w/v. After the polymer was dissolved, MWNTs (2% w/w) were added. The TPU/CNT solution was stirred for an additional 24 hours. For electrospinning, the TPU/CNT solution was injected at a flow rate of 0.5 mL/h and exposed to a 15 kV voltage. The electrospun mat was vacuum-dried and sterilized before cell culture. The mechanical properties of the TPU/CNT scaffold were studied using a micro-tensile machine. Surface structure of the nanofibers was observed using a scanning electron microscope (SEM). SEM images also demonstrated the adherence of rat mesenchymal stem cells (rMSCs) on the nanoporous surface of the electrospun nanocomposite. rMSCs had been obtained from the National Cell Bank of Iran. The viability of cells cultured on the electrospun mat was evaluated by MTT assay.
Results: Addition of CNTs to the nanofibrous cell culture scaffold has improved the elastic modulus and mechanical properties of the elastomeric scaffold as well as its cell affinity, through different mechanisms such as binding of bioactive molecules to the rough and hydrophilic surface of the nanoparticles. The viability of rat mesenchymal stem cells cultured on the nanofibrous mat was more than 90%.
Conclusion: An electrospun nanofibrous scaffold was produced by incorporation of CNT in TPU solution. The mechanical properties, morphology and cell viability of the electrospun mat was studied. TPU/CNT nanocomposite scaffold seems to be an appropriate candidate for application in biological environments, as it exhibits admirable strength and surface elasticity as well as nontoxicity and cell compatibility.
Keywords: Thermoplastic Urethane (TPU), CNT, Scaffold, Electrospinning, Tissue Engineering

Ps-88: The Biological Properties of Chitin Scaffold for Bone Tissue Engineering

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Objective: These days because of bone defects the people who need bone graft are increasing. Autograft is the gold standard method for bone regeneration. However because of some reasons such as the risk of inflammation in donor site, the use of this method is restricted. Therefor scientist proposed bone tissue engineering as an alternate method in order to eliminate this kind of limitations. Scaffold is one of the main component of bone tissue engineering which should have some specific properties. Chitin is the second most abundant natural polymer in the world which is completely biocompatible and could be used as a scaffold for bone tissue engineering. Freeze-drying as fabrication method is widely used in bone tissue engineering because of its unique properties. In this study chitin as a biopolymer was extracted from shrimp shell in order to use for bone tissue scaffold.
Materials and Methods: MTT assay and DAPI staining was utilized for investigation the biocompatibility and cytotoxicity of the scaffolds. MTT assay was performed 1, 3, 5, 7 and 14 days after culture.
Results: The obtained results from chitin/chitosan scaffold was compared with results gained from pure chitosan scaffold (the biocompatibility of chitosan has been proved in numerous studies). The results showed that this scaffold is biocompatible and non-cytotoxic for human mesenchymal stem cells.
Conclusion: Thus this scaffold could be a great candidate for using in bone tissue engineering.
Keywords: Extracted Chitin, Freeze-drying, Human Mesenchymal Stem Cells, Biocompatibility Assay, Bone Tissue Engineering

Ps-89: The Synergic Effect of Low-Level Laser Therapy and Human Bone Marrow Mesenchymal Stem Cell-Conditioned Medium on The Contraction of Healed Open Skin Wound in Diabetic Rats

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Objective: The nobility of this scientific work was to analyze the amalgamate effects of pulsed wave low-level laser therapy (PWLLLT) and human bone marrow mesenchymal stem cell-conditioned medium (hBM-MSCs- CM) on the wound contraction in an animal prototype for type I diabetes mellitus (DM). PW LLLT manifested bio stimulatory sequel on wounds in diabetic mammals. Secretomes was administered into the contusions by the appliance of BM-MSCs CM.
Materials and Methods: Type I DM was induced in rats by the usage of streptozotocin (STZ). Bifurcated creases were performed on proximal and distal segments in dorsal region of the animals. Rats were splitted into four groups. The first group was considered as the control group; the second group, received hBM-MSCs-CM; the third group, received PWLLLT; and the fourth group, received both hBM-MSCs-CM and PWLLLT. CM was collected and concentrated ×20-fold by lyophilized drying and was administrated twice intraperitoneally at the time of surgery and 24 hours after wounding. The proximal wounds in the 3rd and 4th groups were treated with a pulsed laser by 890 nm wavelength, 80 Hz frequency, and 0.2 J/cm^2 energy densities six days per week. On the 15th day, standard image of each wound were taken and the wound closures in the groups were evaluated for wound closure. The data were analyzed by using chi-square test.
Results: In the 3rd (hBM-MSCs- CM) and 4th groups (PW LLLT+ hBM-MSCs- CM), the numbers of wound closures were significantly augmented in the proximal section, compared to the control ones.
Conclusion: It was splendid that PW LLLT alone or in combination with hBM-MSCs- CM significantly accelerated wound closure in the experimental model for STZ-induced type I DM rats.
Keywords: Diabetic Ulcers, Human Bone Marrow-Derived Mesenchymal Stem Cell, Conditioned Medium, Low-Level Laser Therapy, Wound Closure

Ps-90: All-Trans Retinoic Acid Preconditioning to Enhance Mesenchymal Stem Cell Homing *In Vitro*

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Objective: Loss of transplanted stem cells and subsequently inadequacy of cells reaching to injured tissue due to absence of relevant cell surface homing receptor and ligands are the scientific stem cell therapy challenges. In this way, stem cell preconditioning with chemical and pharmacological agents has been shown to increase migration of mesenchymal stem cell and their therapeutic efficacy. Therefore, in the current study, we sought to explore effectiveness of all-trans retinoic acid (ATRA) on increased migration of mesenchymal stem cell (MSCs) *in vitro*.
Materials and Methods: MSCs were treated with various concentrations of ATRA and compared to untreated MSCs for expression of C-X-C chemokine receptor type 4 (CXCR4), C-C chemokine receptor type 2 (CCR2), enzyme matrix metalloproteinase 2/9 (MMP2/9) using qRT-PCR. Enzyme activity of MMP-2/-9 and MSCs migration were assessed using zaymography and scratch test.
Results: In ATRA treated MSCs, expression of CXCR4 ($P < 0.05$), CCR2 ($P < 0.05$), MMP-2 ($P < 0.001$) and MMP-9 ($P < 0.001$) were increased compared to control groups. Elevation of all these genes were reversed by celecoxib (50 μM), a selective COX-2 inhibitor. Activity of MMP-2 ($P < 0.01$) MMP-9 ($P < 0.001$) and MSCs migration was enhanced by ATRA conditioning, in these cells compared to the control group ($P < 0.001$).
Conclusion: Our results revealed that preconditioning of MSCs with ATRA could increase homing of MSCs through affecting some chemokine receptors as well as proteases involved and eventually improving the efficacy of cell therapy.
Keywords: Stem Cell Therapy, All-Trans Retinoic Acid, Migration, Chemokine Receptor, Matrix Metalloproteinase

Ps-91: Let-7a Overexpression Can Induce the Differentiation of Conjunctiva Mesenchymal Stem Cells into Photoreceptor-like Cells

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Objective: Retinal degeneration disorders can lead dysfunction of pigment epithelial cells overlying the photoreceptors and consequently lead visual loss. Currently, there is no effective therapy for retinal degeneration diseases. Cell therapy is a progressing approach for treatment of these conditions. Observations of a recent study demonstrated the differentiation of conjunctiva mesenchymal stem cells (CJMSCs) to photoreceptor-like cells on nanofibrous scaffolds and suggested their potential for cell replacement in retinal regeneration applications. Previously, it has reported that conditional deletion of Dicer inhibits the progression of early progenitors to generate late cell types, suggesting that specific microRNAs (miRNAs) are required for this developmental transition. Recent study has demonstrated that in retinal progenitors, let-7a is one of the key regulators in early to late developmental transition. It has shown that let-7a expression increases over the period of retinal neurogenesis by >32-fold.
Materials and Methods: In this study, we investigated the effect of let-7a overexpression in induced differentiation of CJMSCs into photoreceptor-like cells. Quantitative real-

time RT-PCR was used to analyze differentiated cells and their expression of photoreceptor-specific genes such as rhodopsin.
Results: Our findings have demonstrated that overexpression of let-7a microRNA can significantly increase the expression of photoreceptor specific genes in CJMSCs.
Conclusion: We have found that overexpression of let-7a microRNA can increase the expression of photoreceptor specific genes in CJMSCs. Our results provide new insight into the molecular mechanisms of neural retina differentiation, suggesting that let-7a overexpression can induce photoreceptor differentiation.
Keywords: Retinal Degeneration, Photoreceptor, Conjunctiva Mesenchymal Stem Cells, Differentiation, Let-7a

Ps-92: In-Silico Investigation about Thymoquinone Effects on Apoptosis Mediated by P53 Dependent and Independent Pathway in Cancer Stem Cells

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Objective: Cancer stem cells (CSCs) are cancer cells (found within tumors or hematological cancers) that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. A few natural compounds are potent anticancer agents that offer a non-toxic means for cancer intervention. Understanding the process leading to the inhibition of carcinogenesis by these compounds require a clear identification of their molecular target. Thymoquinone (TQ), a component of black seed essential oil, is known to induce apoptotic cell death and oxidative stress. The molecular pathways of TQ action are not clear completely. Nevertheless, TQ is known to induce apoptosis by P53-dependent and P53-independent pathway in cancer cell line.
Materials and Methods: For in silco work, first thymoquinone was drawn by ChemDraw Ulter software and P53 and P53-independen signaling pathway protein pdb structure was made by Modeller online software and some proteins was taken from protein data bank (www.rcsb.org), and docking was done by Auto Dock software, at the end results analyzed by chimera1.7 viewer.
Results: Thymoquinone molecule interacted with Thr 155, Pro 152 and Gly154 in P53 proteins with followed features, energy binding -4.5, intermol-energy – 4.8 and etc. Also thymoquinone interacted with P53-independent pathway proteins with special properties that have shown thymoquinone can interacted with P53-independent pathway proteins.
Conclusion: TQ interacts with P53 apoptosis pathway better than P53-independent. According to the in silico data, it has been suggested that TQ trigger apoptosis in all type of cancer cells especially in cancer stem cells when interacted with P53 molecule.
Keywords: Apoptosis, Docking, Thymoquinone, P53 Molecule, Cancer Stem Cells

Ps-93: Evaluation of CXCR4 Expression as A Putative CSC Marker in Renal Cell Carcinoma: A study using Tissue Microarrays (TMA)

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Objective: Cancer stem cells (CSC) represent a population with tumor-initiating, self-renewal, and differentiation potential. The aim of this study was to evaluate CXCR4 expression as a putative CSC marker in Renal Cell Carcinoma (RCC).
Materials and Methods: The expression of CXCR4 in specimens from RCC patients was evaluated by immunohistochemistry on a tissue microarray(TMA).One hundred and seventy-three consecutive patients treated surgically for renal cell carcinoma (RCC) between 2010 and 2015 including 106 (61.3%) Clear Cell Renal Cell Carcinoma (ccRCC), 35(20.2%) Papillary and 32 (18.5%) Chromophobe were selected. The association between expression of this marker and tumor characteristics was then analyzed.
Results: The mean expression of CXCR4 is significantly different in RCC subtypes ($P< 0.000$). Increased expression of CXCR4 was significantly correlated with higher grade tumors ($P< 0.000$) and increase of stage ($P = 0.01$). Significant association was found between expression of CXCR4 and Venus invasion ($P= 0.03$). NO significant association was found between expression of CXCR4 and other clinicopathologic features of patients including invasion to pelvis, vein, lymph node and tumor size.
Conclusion: These findings suggest that CXCR4 can be considered as a valuable tool for the study of renal CSCs and provide a therapeutic target for treatment of the patients with renal cell carcinoma in combination with conventional therapy.
Keywords: CXCR4, Cancer Stem Cells (CSCs), Renal Cell Carcinoma, Tissue Microarray (TMA)

Ps-94: Differential Expression of Transcription Factor OCT4 in Renal Cell Carcinoma

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Objective: Cancer stem cells (CSCs) are characterized by self-renewal, resistance to apoptosis and conventional therapies. The aim of this study was to evaluate OCT4 expression as a stemness marker in Renal Cell Carcinoma (RCC).
Materials and Methods: The expression of OCT4 in specimens from RCC patients was evaluated by immunohistochemistry on a tissue microarray (TMA). One hundred and forty-two consecutive patients treated surgically for renal cell carcinoma (RCC) between 2010 and 2015 including 87 (61.3%) Clear Cell Renal Cell Carcinoma (ccRCC), 28(19.7%) Papillary and 27 (19%) Chromophobe were selected. The as-

sociation between OCT4 expression and tumor characteristics was then analyzed.
Results: The mean expression of OCT4 is significantly different in RCC subtypes (P< 0.000). According to nuclear staining, the highest mean of OCT4 H-score was observed in papillary subtype (261.74) followed by chromophob (210.74) and ccRCC (159.59). The statistically significant association was found between expression of OCT4 and invasion to pelvis. No statistically significance association was found between expression of OCT4 and other clinicopathologic features of patients.
Conclusion: These findings suggest that OCT4 can be considered as a valuable tool for the study of renal CSCs and distinguish of various RCC subtypes. However, further studies are required to elucidate the role of OCT4 in carcinogenesis of the tumor.
Keywords: OCT 4, Cancer Stem Cells (CSCs), Renal Cell Carcinoma, Tissue Microarray (TMA)

Ps-95: Study of Nanog Expression in Various Histological Subtypes of Renal Cell Carcinoma

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Objective: Nanog is a key transcription factor in self renewal and pluripotency of embryonic stem cells. The aim of this study was to evaluate Nanog expression as a stemness marker in Renal Cell Carcinoma (RCC).
Materials and Methods: The expression of Nanog in RCC specimens was evaluated by immunohistochemistry on a tissue microarray(TMA). One hundred and fifty-three consecutive patients treated surgically for renal cell carcinoma (RCC) between 2010 and 2015 including 97(63.4%) Clear Cell Renal Cell Carcinoma (ccRCC), 28(18.3%) Papillary and 28(18.3%) Chromophobe were selected. The association between expression of this marker and tumor characteristics was then analyzed.
Results: The mean nuclear expression of Nanog is not significantly different in RCC subtypes (P=0.07). A significant correlation was observed between H-Score of Nanog and RCC subtypes (P=0.005). the highest mean H-Score of Nanog (96.8) was observed in ccRCC. The statistically significant association was not found between expression of Nanog and other patients, clinicopathologic features.
Conclusion: These findings suggest that nuclear staining of Nanog cannot be considered as a valuable tool for the study of renal CSCs. Due to its expression in both cytoplasm and nucleous of RCC subtypes, more data are needed to confirm the correlation of Nanog with the clinicopathological characteristics of RCC.
Keywords: Nanog , Cancer Stem Cells (CSCs), Renal Cell Carcinoma, Tissue Microarray (TMA)

Ps-96: Diabetes Induced Infertility in Rats; Evidence for GDNF, GFRα1 and Bcl-6 Genes Expression

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Objective: The spermatogenesis is known as adult stem cell-dependent process, which is supported by spermatogonial stem cells (SSCs) self-cell-renewal, proliferative expansion, meiosis as well as cytodifferentiation. Diabetes has been reported to negatively affect the spermatogenesis and sperm count in animals and human models. Therefore, present study was done in order to analyze the diabetes-induced alterations at glial cell line–derived neurotrophic factor (GDNF), GPI-anchored binding molecule (GDNF) family receptor alpha 1 (Gfrα1) and Bcl-6 mRNA levels as main genes involving in SCCs renewal system.
Materials and Methods: For this purpose, 24 mature male Wistar rats were divided in to control (with no treatment) and diabetes-induced (streptozotocin-administrated, 40 mg/kg) groups. Following 20, 45 and 60 days after diabetes induction, the mRNA levels of GDNF, Gfrα1 and Bcl-6 were analyzed by using reverse transcriptase PCR (RT-PCR). Moreover, the caspase-3 expression was estimated by using RT-PCR and Immunohistochemical staining.
Results: RT-PCR analyses showed that diabetes significantly (P<0.05) reduced the mRNA levels of GDNF and Gfrα1 following 60 days. The mRNA level of Bcl-6 was significantly (P<0.05) diminished after 20 days in diabetes-induced animals, while it was significantly (P<0.05) increased following 40 and 60 days. Finally, the spermatogonial cells in diabetes-induced groups exhibited a significant (P<0.05) enhancement in caspase-3 expression versus control ones.
Conclusion: Our data showed that, diabetes by down-regulating GDNF and Gfrα1 expression affects SCCs renewal process, which in turn diminishes cellular survival via enhancing caspase-3 expression. Finally, we showed that, diabetes at early stages diminishes Bcl-6 expression, which then increases by the time.
Keywords: Diabetes, Self-renewal, GDNF, Gfrα1, Bcl-6

Ps-97: Implications of Stem Cells for Breast Cancer Therapy

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Objective: Stem cells are found in numerous tissues of the body and play a role in tissue development, replacement, and repair. Mesenchymal stem cells (MSCs) are a heterogeneous mix of stromal stem cells that can give rise to cells of mesodermal lineages, adipocytes, osteocytes, and chondrocytes. They can home to sites of injury where they promote the repair and regeneration of damaged tissues. MSCs also home to sites of tumorigenesis. MicroRNAs (miRNAs) are a class of endogenous non-protein-coding RNAs acted as impor-

tant regulatory molecules by negatively regulating gene and protein expression MiRNAs have been implicated to control a variety of cellular, physiological, and developmental processes. Aberrant expressions of miRNAs are connected to human diseases such as cancer.
Materials and Methods: A comprehensive search review of the MEDLINE and google scholar literature published from 2007 to 2016 which Checked the key words such as stem cells, Breast cancer, microRNAs, Gene therapy, umor progression, tumor suppressors.
Results: Emerging evidence demonstrates that miRNAs are involved in cancer stem cell dysregulation. Recent studies also suggest that miRNAs play a critical role in carcinogenesis and oncogenesis by regulating cell proliferation and apoptosis as oncogenes or tumor suppressors, respectively. Therefore, molecularly targeted miRNA therapy could be a powerful tool to correct the cancer stem cell dysregulation. Also linking MSCs to inflammatory processes and breast cancer development showed their newly discovered physiological roles in the context of the tumor microenvironment. Both are very closely associated with embryonic signaling pathways. Investigated the role of Notch in a pre-invasive breast lesion, ductal carcinoma in situ (DCIS), and have found that aberrant activation of Notch signalling is an early event in breast cancer. High expression of Notch 1 intracellular domain (NICD) in DCIS also predicted a reduced time to recurrence 5 years after surgery. A γ-secretase inhibitor, DAPT, which inhibits all four Notch receptors and a Notch 4 neutralising antibody were shown to reduce DCIS mammosphere formation, indicating that Notch signaling and other stem cell self-renewal pathways may represent novel therapeutic targets to prevent recurrence of pre-invasive and invasive breast cancer.
Conclusion: Stem cell self-renewal pathways such as Notch may represent novel therapeutic targets to prevent recurrence of pre-invasive and invasive breast cancer. the interrelationship between CSCs, embryonic signaling pathways, and EMT/MET offers a continuum of potential therapeutic targets. Knowledge of this relationship is important for both the researcher and clinician in developing and administering optimal therapies for breast cancer patients.
Keywords: Mesenchymal Stem Cells, Breast Cancer, MicroRNAs

Ps-98: Regulation of Th17 Cell Activation by The Decoy Receptor and Mesenchymal Stem Cells During Neuroinflamation

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Objective: Th17 cell is a subtype of T cells involved in chronic inflammatory/autoimmune mediated diseases. Development of an autoimmune response in inflamatory diseases requires activation of Th1- cell and differentiation to effector Th17 cells . Interleukin 23 as a pro-inflammatory cytokine is necessary for the differentiation and activation of Th17 cell. Animal models of chronic inflammatory diseases such as demyelinating diseases strongly demonstrate this cytokine can cause immune response and chronic inflammation. Thus, Interleukin 23 seems to be a therapeutic approach in inflammatory diseases.
Materials and Methods: We Target p19 of Interleukin 23 by engineered mesenchymal stem cell and Targeting , p19 as the specific subunit of Interleukin 23 considered as a realistic therapeutic target for alternative treatments in chronic inflammatory diseases such as multiple sclerosis.
Results: Here we demonstrate that neutralization of interleukine 23 by engineered mesenchymal stem cells promotes regulatory T-cell activation and can causes supression of effector Th17 cell -specific differentiation in Animal models of experimental autoimmune encephalomyelitis (EAE).
Conclusion: This study suggests a central roles of modified mesenchymal stem cell suppressing Interleukin 23 in regulating Th17 lymphocytes differentiation and establish supression of Interleukin 23 by p19 as a regulator of the Th17 signalling axis.
Keywords: Cytokine, Interleukin-23, Th17, Mesenchymal Stem Cell

Ps-99: Optimization of Direct Cardiac Reprogramming from Human Fibroblasts

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Objective: Heart diseases are the most significant cause of morbidity and mortality in the world. Cell therapy has been proposed as a promising approach to treat the cardiac diseases. One of the newest methods to produce human cardiomyocytes required in cell therapy is direct cardiac reprogramming, also known as transdifferentiation, in which non-cardiac cells are reprogrammed to cardiomyocytes by forced expression of cardiac-specific transcription factors (TFs) or microRNAs. There are only few reports in human cardiomyocyte generation using direct reprogramming. However, these reports have used different combination of TFs but their efficiencies remain very low. Finding the network controlling cardiac identity and then stablishing this network in fibroblast can improve the efficiency of direct cardiac reprogramming. Here, we analyzed the gene expression of cardiac-fibroblast (CF), induced cardiomyocyte (iCM) and adult cardiomyocyte (CM) to find new regulators (TFs, miRNAs and signaling pathway) for direct cardiac reprogramming of human cardiac fibroblasts.
Materials and Methods: Microarray libraries GSE49192 were downloaded from NCBI database and analyzed using GEO2R software. The up-regulated and down-regulated genes in cardiomyocytes (CM and iCM) were determined as cardiac specific and fibroblast specific genes, respectively. Protein kinases, transcription factors (TFs), protein-protein interaction network and microRNAs were analyzed using X2K ,ChEA, Genes2Networks, TargetScan-microRNA soft-

ware and database respectively.
Results: Our results predict that overexpression of six cardiac specific TFs including ESRRB, MYC, MEF2A, ESR1, ATF3 and GABPA can improve the efficiency of direct cardiac reprogramming. Also, overexpression of three cardiac specific microRNAs (miR-224, miR-374, miR-124A) might accelerate the reprogramming process by suppressing the hubs of pathways in CF and down-regulated genes.
Conclusion: Combination of six TFs (ESRRB, MYC, MEF2A, ESR1, ATF3, GABPA) and three miRNAs (microRNAs-224, miR-374,miR-124A) might be the most optimized combination for efficient reprogramming of human fibroblasts to cardiomyocytes *in vitro*.
Keywords: Direct Cardiac Reprogramming, Cardiomyocyte, Fibroblasts, Systems Biology

Ps-100: Enrichment of Cancer Stem-Like Cells From Colorectal Cancer Cell Line HT29 Using Lentiviral Vector Carrying E-Cadherin shRNA

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Objective: The cancer stem cell (CSC) hypothesis posits CSCs are a subpopulation of cells within a tumor that can self-renew, drive tumor growth, relapse and metastasis and are resistant to many current anticancer treatments. The better under¬standing of CSCs, may facilitate prevention and treatment of cancers. Epithelial-mesenchymal transition (EMT) plays a crucial role in the differentiation of multiple tissues and organs, and is a key developmental program that is often activated during cancer invasion and metastasis. During the process of EMT, epithelial cells undergo a phenotypic switch, giving rise to a fibroblastoid pheno¬type. The induction of EMT in normal and cancer cell populations renders them more resistant to chemotherapeutic drugs. One protein prominently associated with tumor invasiveness, metastatic dissemination, and poor patient prognosis is the epithelial cell adhesion molecule E-cadherin. E-cadherin plays an important role in maintaining the structural integrity of epithelial sheets. E-cadherin is an important molecule in cancer progression and EMT process. A critical molecular feature of EMT is the down regulation of E-cadherin. The inhibition of E-cadherin expression has been reported in several types of cancers, including: Colorectal cancer (CRC). The aim of study was to create a model of CSC enrichment via the induction of EMT in CRC cell line for cancer study, especially for screening anti-CSC chemotherapeutic drugs. We examined whether the knockdown of E-cadherin leads to significant EMT-like alterations and acquirement of most the properties of CSCs.
Materials and Methods: We performed E-cadherin knockdown in the HT29 CRC cell line using Lentiviral Vector Carrying shRNA. Then we analyzed properties and markers of CSCs , epithelial and mesenchymal cells compared to control cells using several technique including: flow cytometry, Real-time PCR, Immunocytochemistry (ICC), western blot, migration assay, Tumorsphere.
Results: We established an EMT model using the HT29 cell line by CDH1 knockdown. Colorectal CSC markers were enriched in the CDH1 knockdown cells. The cells exhibited mesenchymal morphology and expressed high levels of EMT-related proteins, which confirmed that these cells had undergone EMT.
Conclusion: CDH1 knockdown cells were highly enriched CSCs and may be used as a stable model for cancer research and anticancer drug screening.
Keywords: Cancer Stem Cells (CSC), Epithelial-Mesenchymal Transition (EMT), E-Cadherin, Lentiviral Vector

Ps-101: Synergistic Effects of Temozolomide and Thymoquinone on Cell Proliferation and Matrix Metalloproteinase 2 and 9 Secretion and Expression

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Objective: Temozolomide is an alkylating agent used as a chemotherapy drug for glioblastoma treatment. Nevertheless, resistance to temozolomide is a major obstacle to successful treatment of this cancer. Matrix metalloproteinases are important factors for successful treatment of glioblastoma. The present study was aimed to examine the effects of temozolomide and thymoquinone alone and in combination on proliferation of U87MG cancer cells and also to evaluate metalloproteinase 2 and 9 expression and secretion levels.
Materials and Methods: The effects of temozolomide and thymoquinone on cells viability were evaluated using trypan blue assay and median effect analysis. CompuSyn software (ComboSyn, Inc., Paramus, NJ, USA) was used for data analysis. Matrix metalloproteinases mRNA expression and protein levels were examined by real-time RT-PCR and ELISA assay, respectively.
Results: Both temozolomide and thymoquinone have a significant dose dependant cytotoxicity effect on U87MG cells and combination of both reveal a synergistic effect with combination index values between 0.51 and 0.33 and the mean combination index about 0.39 for all tests. This combination also results in a dose reduction for both temozolomide and thymoquinone, and reduces the IC50s to about 1.53 folds smaller for thymoquinone and 2.68 folds smaller for temozolomide. Both MMP 2 and 9 were downregulated by TMZ and/or TQ treatment significantly.
Conclusion: We suggest that the combination of temozolomide and thymoquinone may emerge as a promising strategy for the successful treatment of glioblastoma multiforme.
Keywords: Glioblastoma Multiforme, Temozolomide, Thymoquinone, Matrix Metalloproteinases

Ps-102: Therapeutic Effect of Adipose Derived Stem

Cells-Conditioned Medium on The 6-Hydroxydopamine Rat Model of Parkinson's Disease

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Objective: Parkinson's disease is a major neurodegenerative disorder characterized by bradykinesis, muscular rigidity and resting tremor. The pathological basis for PD is a deficiency of dopamine in the striatum, resulting from the loss of dopaminergic neurons. Adipose tissue-derived stem cells (ADSCs) have many advantages for cell therapy because of the easy availability and pluripotency without ethical problems. Here we want to evaluate the effects of intravenous transplanted ADSCs and conditioned medium (CM) on motor impairment, of rat Parkinsonian models.
Materials and Methods: Parkinson model was constructed by the unilateral lesion of striatum of male Wistar ratsusing 20µg of 6-hydroxydopamine (6-OHDA) as lesion group. Cell and CM groups were lesioned animals that received intravenous injection of 3×10^6 cells and conditioned medium repectively. α-MEM group was injected with medium instead of cell.
Results: then,the level of SOD,GPX,MDA was measured. the activity of GPX&SOD showed significant increase in the treatment group compared to the injury group.however,the level of MDA in the groups of cell and conditioned medium decresed significantly compared to groups pf injury and α-MEM.
Conclusion: then,the level of SOD,GPX,MDA was measured.the activity of GPX&SOD showed significant increase in the treatment group compared to the injury group. however,the level of MDA in the groups of cell and conditioned medium decresed significantly compared to groups pf injury and α-MEM.
Keywords: Parkinson, Adipose Drived Stem Cells, 6-OHDA, Conditioned Medium

Ps-103: The Cytotoxic Effects of Cisplatin on Mouse Acute Lymphoblastic Leukemia and Spermatogonial Stem Cells *In Vitro*

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Objective: Testicular cancer is the most common cancer affecting men in reproductive age, and cisplatin is one of the major helpful chemotherapeutic agents for treatment of this cancer. In addition, exposure of testes cancer cells to cisplatin could potentially eliminate tumor cells from germ cells in patients. The aim of this study was to evaluate the effect of cisplatin on viability of mouse acute lymphoblastic leukemia cell line (EL-4) and neonatal mouse spermatogonial cells *in vitro*.
Materials and Methods: In this study, the isolated spermatogonial stem cells (SSC) and EL-4 were divided into six groups including Control (received Medium), Sham (received DMSO in medium) and Experimental groups which received different doses of cisplatin (0.5, 5, 10, 15µg/ml). Cells viability was evaluated with MTT assay. The identity of the cultured cells was confirmed by the expression of specific markers.
Results: Our finding showed that viability of both SSC and EL-4 cells was reduced with the dose of 15µg/ml when compared to the control group ($P\leq0.05$). Also, the differences between the IC50 in doses 10 and 15µg/ml at different time were significant ($P\leq0.05$). The number of TUNEL-positive cells was increased, and the BAX and Caspase3 expressions were up-regulated in EL4 cells for group that received an effective dose of cisplatin.
Conclusion: Despite the dramatic effects of cisplatin on both cells, spermatogonial stem cells could form colony in culture.
Keywords: SSCs, Cisplatin, EL4 Cells, Viability, TUNEL Assay

Ps-104: Delivery of MiRNA to Glioma Cancer Cells by Warton's Jelly-Derived Mesenchymal Stem Cells and Its Subsequent Effects in Deacrease of Cell Growth and Migration

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Objective: Glioma is considered as one of the most aggressive and lethal human brain tumors. Detailed studies have revealed that this kind of tumor is resistant to conventional therapies. Accumulating data indicate that miRNAs are involved in advanced stages of cancer progression and may act as activators or suppressors of tumorigenesis. Previous studies report that level of miR-124 is downregulated in glioma specimens. In glioblastoma, miR-124 acts as a tumour suppressor. Previous studieshave also indicated that Mesenchymal Stem Cells (MSCs) have a tumor tropic property and therapeutics effects. It is further shown that the secretom of warton'sJelley MSCs (WJ-MSCs) are capable of promoting the neuronal differentiation.
Materials and Methods: The aim of this study was to use WJ-MSCs as a vehicle for delivery of exogenous miR-124 to glioma cancer cells.we co-cultured WJ-MSCs, that transduced

by miR-124, with U87 cell line as model of human glioma cancer. The effects of exogenous miR-124 on glioma cancer phenotype were examined by qPCR, migration assay, MTT and Anexin V methods.
Results: It is shown that the delivered miR-124 significantly decreased the expression of CDK6 genes and increased the expression of P53, P16, Smad4 and caspase-3 genes. Furthermore, migration assay, MTT and anexin V studies showed that delivery of miR-124 by WJ-MSCs,decreases the migration and proliferation of U87 cells.
Conclusion: Our results revealed thatWJ-MSCs can deliver therapeutic exogenous MiRNA to cancer cells with clinical implications.
Keywords: Glioblastoma, miR-124, WJ-MSCs, Migration, Cell Proliferation

Ps-105: Comparative Growth Rate of Equine Abdominal versus Gluteal Fat-Derived Mesenchymal Stem Cells

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Objective: It is established that mesenchymal stem cells (MSCs) derived from various sources in addition to the similarities; they have some differences in the characteristics such as growth rate. Aim of this study was to compare growth rate of equine adipose-derived MSCs (AMSCs) which were collected from two different adipose tissues (abdominal versus gluteal).
Materials and Methods: AMSCs were isolated from two regions including gluteal (tail base) and abdominal region of a 1.5 aged euthanized horse. AMSCs were enzymatically isolated and cultured until passage 3 (P3). Then P3 cells were analyzed for MSC characteristics. To evaluate cell growth rate, AMSCs at P3 were seeded in 12-well plates with a density of 30,000 cells per well .The cells of 3 wells were daily trypsinized and counted until day 8. Finally, the growth curve of cells in both groups was plotted.
Results: Gluteal region was an easily accessible site for fat collection and fewer vessels were in gluteal region which makes easier the fat collection process. AMSCs isolated from 2 different regions showed typical characteristics of MSCs including expression of specific markers (CD29, CD90 and CD44) as well as trilineage differentiation. Growth curve indicated that growth rate of AMSCs of gluteal region is more rapid compared with AMSCs derived from abdominal region. So, gluteal fat-derived AMSCs need less culture time *in vitro*.
Conclusion: We found that equine gluteal fat-derived AMSCs have the better growth rate than abdominal derived AMSCs and they are a more suitable choice for cell therapy in equine medicine.
Keywords: Mesenchymal Stem Cell, Growth Rate, Equine, Adipose

Ps-106: Decellularization of Equine Digital Flexor Tendon as a Suitable Scaffold for Tissue Engineering

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Objective: Tendon injuries are major cause of orthopaedic injuries in athletic horses. Tissue engineering (TE) provides an alternative method for managing these disorders. One of the major challenges in TE is the fabrication of a suitable scaffold that can provide proper niche for cells (such as mesenchymal stem cells) and three- dimensional structure. Among biological scaffolds, the use of decellularized tendons represent a good approach to regenerative medicine. Thus, this study aimed to decellulrize equine superficial digita flexor tendon (SDFT) for obtaining an efficient scaffold.
Materials and Methods: Equine SDFT was aseptically harvested from the forelimb of a 1.5 aged horse which euthanized for conditions unrelated to musculoskeletal disease. 10 cm pieces in length of tendon were divided into 2 groups as control and treatment. Samples was decellularized using 4 freeze-thaw cycles followed by 48h incubation in 1 M Tris–HCl (pH 7.8) combined with 1% sodium dodecyl sulfate (SDS), 10 mg/ml DNase-I under continuous agitation at 4˚C. Samples were embedded in paraffin, longitudinally sectioned into 5 µm slices, and stained with H&E and Masson's trichrome to assess cells and morphological extracellular matrix.
Results: The histological results demonstrated very few visible nuclei in treatment group compared with control along with a small increase inporosity and maintenance of collagen content following decellularization. This condition provides a physical environment similar to native tendon with minimal residual cellular debris which can be used as a therapeutic graft material.
Conclusion: The results showed that the modified freeze-thaw method could provide a decellularized SDF tendon which can be used as a suitable scaffold in equine tenogenic differentiation studies and for treatment of tendon disorders.
Keywords: Decellularization, Superficial Digital Fexor Tendon, Tissue Engineering, Equine, Mesenchymal Stem Cell

Ps-107: Evaluation of KDM5A Knockdown by shRNA in Acute Myeloid Leukemia Cell Line

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Objective: Acute Myeloid Leukemia (AML) is a hemato-

logic malignancy that originates from hematopoietic stem and myeloid progenitor cells .AML is the most common type of acute leukemia diagnosed in adults and the second most common leukemia in children. Histone lysine demethylases (KDMs) have been nicknamed "erasers" for their ability to remove methyl groups from histone substrates play important role in homeostasis and cancer process. In cancer cells, KDMs can activate or repress gene transcription. Lysine-specific demethylase 5A plays critical roles in controlling transcription and chromatin architecture, yet its biological function largely remain uncharacterized, particularly in the context of human cancer. It has been reported that KDM5A gene was significantly amplified and over-expressed in various human tumors, including breast cancer, gastric cancer, cervical cancer and etc. Over-expression of KDM5A was associated with breast cancer and lung cancer drug resistance. In the present study, down regulation of KDM5A was performed in acute myeloid leukemia cell line (HL60) to investigate the effect of its reduced expression in AML.

Materials and Methods: Short hairpin RNA (shRNA) was designed against KDM5A and inserted to lentiviral vector for virus packaging. Transduced HL60 cell line then, cell behaviors were investigated using MTT assay, flow cytometry and Annexin V then the expression of several genes involved in leukemogenes and resistance to therapies was investigated by QRT-PCR.

Results: we found that reduced expression of KDM5A results in down regulation of SOX4, JAK2, STAT3, CREB and MAPK1 which play important roles in tumorigenesis of AML, also KDM5A as key role to induce apoptosis in HL60 cells.

Conclusion: KDM5A knock down by shRNA plays important role in apoptosis pathways and tumorgenesis of AML, therefore we suggest that KDM5A could be new goal for AML therapy.

Keywords: KDM5A, AML, Epigenetic, Cancer, Leukemia

Ps-108: Non-Motor Symptoms in Parkinson's Disease and Efficacy of Treatment in a Complex Therapy Using Fetal Stem Cells

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Objective: To evaluate the dynamics of non-motor symptoms (NMS) by scores in the patients with Parkinson's disease during complex treatment using fetal stem cells (FSCs) obtained from human fetuses with 5-10 weeks of gestation.

Materials and Methods: A comparative study for 63 patients with Parkinson's disease (PD) suffering from NMS with various degree of clinical presentation has been performed to identify the effect of combined treatment on the quality of life, cognitive functions, sleep and extent of depressive disorders in patients. The main group (MG) consisted of 32 patients, who apart from standard therapy underwent treatment using suspensions of FSCs harvested from the fetal liver and brain. The control group (CG) included 31 patients. The patients in both groups have been compared according to their sex and age.

Results: Significant decrease of NMS in patients of the MG was reported 6 and 12 months after treatment. This value was significantly lower compared to the patients in the CG. By means of a detailed evaluation, significant improvement in quality of the objective parameters of sleep, daily activities and decrease of depressive disorders were reported in the MG. The treatment results were significantly higher in the MG if compared to those in the CG over 1 year after therapy.

Conclusion: FSCs therapy proved to induce positive effects on both subjective and objective manifestations of PD by improving the patient's quality of life when included into the standard scheme of treatment for PD patients with developed NMS.

Keywords: Parkinson's Disease, Non-Motor Symptoms, Fetal Stem Cells

Pas-109: Fetal Stem Cell Transplantation in Autism Spectrum Disorders

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Objective: Autism spectrum disorders (ASDs) are heterogeneous complex neurodevelopmental pathologies characterized by abnormalities in social interactions, with the presence of impaired communication, restricted interests, and repetitive stereotypic behaviors. Despite extensive research efforts, there are still no agreed upon clinical management of this difficult disorder. Based on recent advances in the understanding of immunological pathologies associated with ASDs, it appears stem cell therapies could be designed to target these disorders. Among stem cell populations, fetal stem cells (FSCs) have immunoregulatory functions found in primordial mesenchymal stem cells, but in addition, exhibit a potent expansion capacity and plasticity, showing a great potential for clinical use. Furthermore, FSCs are potent and implantable "biopharmacies" capable of delivering immunoregulatory and trophic signals to the host.

Materials and Methods: This study investigated the safety and efficacy of FSC transplantations in treating autistic children. Subjects were monitored at pre, and then 6 and 12 months following the transplantations which consisted of two doses of intravenously and subcutaneously administered FSCs. Autism Treatment Evaluation Checklist (ATEC) test and Aberrant Behavior Checklist (ABC) scores were performed. Laboratory examinations and clinical assessment of adverse effects were performed in order to evaluate treatment safety.

Results: No adverse events of significance were observed in ASD children treated with FSCs, including no transmitted infections or immunological complications. Statistically significant differences were shown on ATEC/ABC scores evaluation regarding speech, sociability, sensory and health, as well as the total score, in treated patients compared to pre-treatment values (time 0) at several time-points (six and

twelve months) post-treatment (P<0.05).
Conclusion: Promising results of this study warrant addition investigations into the mechanisms of cell therapies for autism.
Keywords: Stem Cells, Autism, Cell Transplantation

Ps-110: Dissection of Regulatory Elements during Direct Conversion of Somatic Cells to Neurons

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Objective: A subtle gene expression modification occurs during direct conversion of somatic cells, specially fibroblasts, to neurons. Although, many aspects of this process have been studied individually by many groups, a combined and data-driven approach has not yet been used to study this approach in details. Besides, most transcription factors and miRNAs involved in this process are not studied.
Materials and Methods: In the current work, the gene expression data obtained from 10 independent reports were combined. These reports cover almost all intermediate steps in direct conversion of fibroblast to neurons. We have also included one data set containing the impacts of the fibroblast age on such conversion. Altogether, 68 data sets were used. Our comparison, using online tools, freely available software and in-house scripts, include different types of nerve cells and fibroblasts at different age. We have analyzed both involved transcription factors and miRNAs. Using such data, we have also constructed regulator networks for each step during cell conversion.
Results: Differentially expressed TFs were identified during conversion of fibroblast to nerve cells. Additionally, miRNAs with a role in this process were also introduced. The constructed network unveiled dynamic changes in the regulatory elements during this conversion.
Conclusion: Such study would be useful in designing more controllable approach in conversion of somatic cells into nerve cells. Besides, with this knowledge, one would be able to produce different types of nerve cells by pausing at intermediate steps during conversion.
Keywords: Nerve Cells, Direct Conversion, Fibroblasts, Regulatory Network

Ps-111: TGF-Beta Signaling Pathway Effects on Cardiac Differentiation of Ovine Mesenchymal Stem Cells

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Objective: Mesenchymal stem cells (MSCs) have potential of self-renewal and differentiation into many cell types and can be used for cells therapy. 5-Azacytidine induces differentiation of MSCs into cardiomyocytes. The mechanism of the 5-azacytidine action on cardiac differentiation from MSCs remains controversial. The aim of this study was to evaluate the effect of 5-azacytidine on differentiation of sheep fetal bone marrow MSCs into cardiomyocytes.
Materials and Methods: Isolated sheep fetal bone marrow MSCs was cultured in DMEM: F12 medium. Passaged-3 cells were examined for their differentiation capacity into osteocytes and adipose cells. Cells after third passage treated with 10 μM 5-azacytidine for 48h followed by medium without 5-azacytidine for twenty-eight days. Expression of cardiac alpha-actinin was analyzed by immunohistochemistry. Expression of cardiac specific genes ANP, Connexin 43 and MYH6 and TGF-beta signaling pathway gene Samd2 were evaluated by Real-Time PCR.
Results: Oil red for adipose cells and alizarin red staining for osteocytes indicated that the cells from all studied groups maintained the differentiation potential into adipose and osteocytes cells. Expression of connexin 43, ANP and MYH6 were also detected by Real-time PCR. Expression of Smad2 was observed by Real-time PCR. Sarcomeric alpha-actinin was also partially detected by immunocytochemistry on these cells.
Conclusion: These results suggested that sustained activationof TGF-beta by 5-Aza contributed to the induction of the differentiation of ovine MSCs into cardiomyocytes *in vitro.*
Keywords: 5-azacytidine, Mesenchymal Stem Cells, Sheep Fetal, Cardiomyocytes Differentiation, TGF-Beta

Ps-112: Determination of MiRNAs Modulate CFIm25 Gene in Breast Cancer

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Objective: Breast cancer is the most commonly recognized cancer in women worldwide. In Iran, it ranks first among cancers diagnosed in women and is the fifth most common cause of death. Recent data have shown therapeutic and prognosis utilization of microRNAs in breast cancer. MicroRNAs constitute a recently discovered class of conserved non-coding RNAs that play key roles in the regulation of gene expression, acting at the post-transcriptional level and may fine-tune the expression of as much as 30% of all mammalian protein-encoding genes. Human Cleavage Factor Im (CFIm) is an essential component of the pre-mRNA 3′ processing complex that functions in the regulation of poly(A) site selection through the recognition of UGUA sequences upstream of the poly(A) site. Recently CFIm25 has been identified as a broad repressor of proximal poly(A) site usage that, when depleted, increases cell proliferation. The aim of this study is to investigate which microRNAs modulate CFIm25 gene in breast cancer.
Materials and Methods: First, potential miRNAs that target CFIm25 were selected using bioanformatic analysis. Then, human breast cancer cell line of MCF-7 was infected

with lentiviruses containing predicted miRNAs precursor sequence. The expression level of selected miRNAs and CFIm25 were estimated by QRT-PCR in 50 tumor and 50 normal tissues. Also, luciferase assays were performed for confirmation of miRNAs binding to CFIm25.
Results: Ectopic expression of predicted miRNAs in MCF7 cell lines dramatically reduced CFIm25 expression. It was found that expression level of predicted miRNAs inversely correlates with CFIm25 level in 50 primary breast samples.
Conclusion: This is the first study which evidently indicates miRNAs correlation with CFIm25 expression in breast cancer cells. Our data suggests oncogenic role for selected miRNAs and presents a rationale for the down regulation of these miRNAs as a novel strategy to improve treatment response in breast cancer.
Keywords: Cancer, CFIm25, MiRNA

Ps-113: Involvement of Long Non-coding RNAs in Multiple Sclerosis

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Objective: Long non-coding RNAs (lncRNAs) are a type of regulatory RNA molecules, longer than 200 nucleotides which are crucial in lots of cellular processes including immunity and inflammation. Multiple sclerosis (MS) is an inflammatory immune-mediated disorder of central nervous system (CNS) which is widely characterized by demyelination and inflammation. In this study we aimed to investigate expression pattern of lncRNAs in peripheral blood mononuclear cells (PBMC) of relapsing-remitting MS (RR-MS) patients. Expression level of chosen lncRNAs in 30 RR-MS patients including 2 groups of patients in relapsing and remitting phases measured by quantitative Real-time PCR compared to 15 healthy volunteers.
Materials and Methods: Relapsing phase patients were with severe attack, and without any consumption of immune modulatory drugs, whereas remitting phase patients were under treatment by immune modulatory drugs. PBMCs were isolated from blood, using density gradient ficoll. Total RNA was extracted and cDNA synthesis was fulfilled. Real-time quantitative PCR reactions were carried out and data analysis was performed by REST 2009 software in ΔΔCT method.
Results: Interestingly results showed some lncRNAs up-regulation in relapse phase of MS patients compared to healthy controls (P value=0.001) and remitting phase of patients (P value=0.001).
Conclusion: The data would seem to suggest that lncRNAs play crucial roles in MS disease progress and can be considered as potential therapeutic targets in order to prevent sever attacks in RR-MS patients. In addition data analysis of this study arguably confirmed some lncRNAs as potential helpful diagnostic biomarkers to distinguish between relapse and remitting phases of MS disease in RR-MS patients.
Keywords: lncRNA, Multiple Sclerosis, Biomarker

Ps-114: The Therapeutic Potential of Human Adipose-Derived Mesenchymal Stem Cells in Experimental Autoimmune Arthritis Treatment

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Objective: Rheumatoid arthritis (RA) is an autoimmune disorder characterized by synovial hyperplasia, inflammation and articular destruction leading to clinically significant functional impairment. Human adipose derived mesenchymal stem cells (hAD-MSCs) possess potent immunosuppression and anti-inflammation effects that make them eligible candidates for RA cell-based therapy. We aimed to assess the therapeutic effect of hAD-MSCs in a collagen-induced arthritis (CIA), a relevant animal model for human rheumatoid arthritis.
Materials and Methods: We have studied three groups including treatment, control and normal (five rats in each group). hAD-MSC were tested in collagen induced arthritis model in Rat. Arthritis was assessed clinically, histologically and by radiography technique. The immunosuppressive properties of hAD-MSCs were indicated by mixed lymphocyte reactions assay *in vitro*. Then, we assessed the effects of intra-articular and intraperitoneal injection of hAD-MSCs in a collagen-induced arthritis (CIA).
Results: Knee joint diameter (swelling) was measured as a clinical indication of joint inflammation. This parameter was significantly less in hAD-MSCs -treated rat compared to untreated animals 18 to 30 days after arthritis induction ($P<0.01$). radiologic and Histopathological changes such as synovium proliferation, inflammatory infiltration, soft tissue swelling, severe cartilage and bone destruction, were significantly improved in hAD-MSCs-treated rat compared to control-treated animals at day30 ($P<0.05$).
Conclusion: Based on our current data, hAD-MSCs injection has therapeutic effect including: reducing inflammation, joint swelling and articular destruction in CIA rat model. Since, hAD-MSCs is easily accessible, culture and also grow rapidly, so they could represent a valuable tool for stem cell-based therapy in chronic inflammatory disease such as RA.
Keywords: Human Adipose- Derived Mesenchymal Stem Cells, Collagen- Induced Arthritis, Anti-Inflammatory

Ps-115: Triggering Differentiation of P19 Embryonic Stem Cells Using Oligodeoxynucleotide Decoy Targeting Nanog Gene

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Objective: Oligodeoxynucleotide Decoy or Transcription Factor Decoys (TFDs) are exogenous oligonucleotides which can compete by cis-elements in promoters or enhancers for binding to TFs and down regulating gene expression in a specific manner. Differentiation of stem cells is an inevitable process in cell therapy. there are some studies that applied ODN decoys to differentiate pre cartilainous stem cells (PSCs) and cd-ODN to differentiate CSCs, we recruited TFDs against stemness master regulators in mouse EC stem cells. Many transcription factors such as Nanog, Oct-4, Sox2, Klf4 and Sall4 act as master regulators in maintenance of stemness. Nanog is a member of homeo domain transcription factor family, overexpression of Nanog maintains ESCs self-renewal in the absence of leukemia inhibitory factor (LIF),and inhibition of Nanog expression leads to endoderm differentiation. the present study is the first report of a TFD approach against a master regulator of stemness Nanog, for down regulation purposes in P19 embryonic carcinoma stem cell.
Materials and Methods: For this porpus different simple decoys against Nanog were designed and purified in HPLC grade. transfection of ODNs to P19 cells was carried by lipofectamine 2000 and gene expertion analysis performed by Real time PCR.
Results: The results showed that the applied decoys decreased the expression of Nanog and downstream genes.
Conclusion: Altogether our results demonstrated that down-regulation of Nanog can cause ESCs differentiation since reduction in Nanog levels is a prerequisite for differentiation.
Keywords: Transcription Factor Decoy, Stemness, P19 Cells, Nanog

Ps-116: cAMP Agonist Increases Dopaminergic Differention of Trabecular Meshwork-Mesenchymal Stem Cells

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Objective: It is well known that elevation of intracellular cAMP result in activation of PKA. It is also shown that activation of PKA result in dopaminergic differentiation of stem cells from various sources. Trabecular meshwork-mesenchymal stem cells (TM-MSC) are multipotent stem cells that can differentiate into different lines of ocular cells such as photoreceptors and conjunctiva, however their potential toward neural differentiation is not known. In the present study, neural differentiation potential of these cells was examined. Also by adding cAMP agonist (db-cAMP) in our differentiation medium, the role of cAMP on dopaminergic differentiation of TM-MSCs was investigated.
Materials and Methods: To address these questions, TM-MSCs were primarily cultured in DMEM containing 20% FBS until they became 80% confluent. Their medium was then changed to DMEM containing 10% FBS and neural differentiation media without db-cAMP (Retinoic Acid 10 Mm, Fibroblast growth factor (FGF) 20 ng/ml , Ascorbic acid 50µM , IBMX 0.5 Mm , FBS %7.5) or neural differentiation factors with db-cAMP (IBMX 0. 5 mM, retinoic acid 10µM and dbCAMP 0. 5 mM). After six days, TM-MSC were examined for neuronal morphology and general neural marker (nestin, beta tubulin I, NSE), early dopaminergic markers (Nurr1), glial marker (GFAP).
Results: Our morphological analysis revealed that TM-MSCs show morphological characteristics of neuron like cells as early as two days of culture. Our RT- PCR results revealed that expression of all neural specific genes, but not glial specific marker, were significantly increased in cells treated with neural differentiation media with db-cAMP in compare to the neural media without db-cAMP.
Conclusion: Altogether our results demonstrated that TM-MSCs have the neural differentiation potential toward early dopaminergic fate and that cAMP signaling is involved in this differentiation pathway.
Keywords: Dopaminergic Differentiation, Trabecular Meshwork-mesenchymal Stem Cells, cAMP

Ps-117: Generation of Functional Hepatocyte-Like Cells from Human Pluripotent Stem Cells in a Scalable Suspension Culture

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Objective: Recent advances in human embryonic and induced pluripotent stem cell-based therapies in animal models of hepatic failure have led to an increased appreciation of the need to translate the proof-of-principle concepts into more practical and feasible protocols for scale up and manufacturing of functional hepatocytes.
Materials and Methods: In this study, we describe a scalable stirred-suspension bioreactor culture of functional hepatocyte-like cells (HLCs) from the human pluripotent stem cells (hPSCs). To promote the initial differentiation of hPSCs in a carrier-free suspension stirred bioreactor into definitive endoderm, we used rapamycin for ‘‘priming’’ phase and activin A for induction. The cells were further differentiated into HLCs in the same system. HLCs were characterized and then purified based on their physiological function, the uptake of DiI-acetylated low-density lipoprotein (LDL) by flow cytometry without genetic manipulation or antibody labeling. The sorted cells were transplanted into the spleens of mice with acute liver injury from carbon tetrachloride.
Results: The differentiated HLCs had multiple features of primary hepatocytes, for example, the expression patterns of liver-specific marker genes, albumin secretion, urea production, collagen synthesis, indocyanin green and LDL uptake, glycogen storage, and inducible cytochrome P450 activity. They increased the survival rate, engrafted successfully into the liver, and continued to present hepatic function (i.e., albumin secretion after implantation).
Conclusion: This amenable scaling up and outlined enrichment strategy provides a new platform for generating functional HLCs. This integrated approach may facilitate biomedical applications of the hPSC-derived hepatocytes.
Keywords: Pluripotent Stem Cell, Differentiation, Hepato-

cytes, Scale- up

Ps-118: Evaluating The Effect of Eugenol on Mouse Bone Marrow Mesenchymal Stem Cells Survival *In Vitro*

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Objective: Stem cells, particularly mesenchymal stem cells (MSCs), and the use of natural ingredients have provided new hopes for the treatment of many human diseases. Eugenol is a natural and versatile vegetable molecule, which has a wide variety of applications in different fields. Despite extensive studies on the biological and pharmacological effects of Eugenol, but there is no report on its effects on stem cells biology. Given the importance of MSCs in immunomodulation and tissue regeneration *in vivo*, as well as their applications in regenerative medicine, and also the pro-/anti-oxidant and immunomodulatory effects of Eugenol, it can suggest that this compound may affect the biology and function of MSCs. The aim of this study was to investigate the effect of Eugenol on mouse bone marrow-derived MSCs survival *in vitro*.
Materials and Methods: MSCs were isolated from 4-8 week mice. Differentiation assays into osteoblasts and adipocytes were used to confirm MSCs identity in cell cultures. The effect of different concentrations of Eugenol (12.5, 25, 50, 100, 200, 400, 800, 1600, 3200µg/ml) on MSCs viability was evaluated by MTT test at 24, 48, and 72h. Moreover, morphological changes of the treated MSCs were examined by microscopic studies, compared with untreated controls.
Results: Our study demonstrated that Eugenol induced a distinct dose- and time- dependent diminution of MSCs viability. The IC50 values of Eugenol on MSCs were determined as 400µg/ml after 24 and 48h, and 200µg/ml after 72h. Furthermore, microscopic observations revealed that Eugenol reduced the number of MSCs in a dose- and time- dependent manner and prominent morphological changes were common after 24h of treatments with 100µg/ml of Eugenol.
Conclusion: Results show that Eugenol had less toxicity on MSCs at low concentrations and it can suggest that this compound may affect proliferation, migration and immunomodulatory properties of MSCs, however, different investigations are required to confirm these effects.

Keywords: Eugenol, Mesenchymal Stem Cell, Survival, MTT Assay

Ps-119: Electrospun Silk Fibroin Nanofibers Containing Hydroxyapatite Nanoparticles for Bone Tissue Engineering

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Objective: Silk fibroin (SF) has been introduced as a good candidate for usage as collagen in bone tissue engineering, because of its prominent biocompatibility and potential. Electrospun scaffolds of SF have recently attracted a great deal of scientific interest, due to their considerable analogy to collagen and high interconnectivity. Application of osteoconductive bioceramics such as Hydroxyapatite (Hap) has been suggested to enhance the biocompatibility and bioactivity of SF nanofibers. Since uniform dispersion of hydroxyapatite in the polymer matrix improves the ultimate properties of nanocomposite, here a good method for impeding the agglomeration of Hap particles has been proposed. In this study, the solubility of Hap nanoparticles in the SF/Formic acid system was examined. Furthermore, several techniques such as FTIR, MTT and SEM were used to characterize the structure and properties of SF/Hap nanofibers.
Materials and Methods: SF/Hap nanofibers were prepared in formic acid (FA). First, Sponge SF was dissolved in FA to obtain a homogeneous solution (13% (w/v)). Then Hap nanoparticles were introduced into the solution such that the mass ratio of Hap to SF was 1 wt%. The voltage, flow rate and the distance between tip and collector were set to 20 kV, 0.5 ml/h and 13 cm, respectively. The morphology of the prepared nanofibers was studied using SEM. SF/Hap nanofiber was studied using a FT-IR spectrometer with a range of wave numbers from 400 to 4000 cm-1 in absorbance mode at room temperature. MG63 cells provided by the National Cell Bank of Iran were examined on days 3, 7 and 14 using the MTT assay. On the 3th day after cell seeding, cell attachment to the electrospun scaffolds was analyzed by SEM.
Results: SEM images of the scaffold were indicative of modification of the nanofibers with nanographene. FTIR spectrum revealed the presence of phosphate group picks of HAp at 528 cm-1 and 575 cm-1. The observed peak at 1630 cm-1 was attributed to silk structure (β-sheet). SEM images indicated an appropriate cell attachment to the surface of electrospun SF/HAp. MTT assay was indicative of the increase in cell proliferation on SF/HAp nanofibers on days 3, 7 and 14 compared to the SF and control samples.
Conclusion: SF/Hap nanofiber was prepared using formic acid. EDX mapping of Ca and P in SF/Hap nanocomposite exhibited a uniform distribution in the polymer matrix. MTT assay revealed that electrospun SF/HAp was more biocompatible for MG63 cells, compared to the SF samples. Consequently electrospun SF/Hap can be used as a good candidate for bone tissue engineering applications.

Keywords: Silk Fibroin, Hydroxyapatite, Electrospinning, Bone Tissue Engineering

Ps-120: Effects of Partial AZFc Microdeletions on Micro TESE Results in Azoospermic Infertile Men

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Objective: Microdeletions of Yq chromosome are the most frequent molecular genetic etiology for the male infertility which usually spans AZFa, AZFb and AZFc regions. Microdeletions are mostly seen in AZFc region and usually cover genes actively involved in spermatogenesis. Partial AZFc microdeletion may also happen with various spans namely gr/gr, b2/b3 and b1/b3. It is known that the micro TESE outcomes as the surgical process for sperm retrieval from the testis in infertile azoospermic men can be predetermined based on the type of AZF microdeletion. Present study was aimed to evaluate the effect of partial AZFc microdeletions on micro TESE results.
Materials and Methods: 200 infertile azoospermic men referred to Royan institute were evaluated for the presence of partial AZFc microdeletions before they undergo micro TESE. Partial AZFc microdeletions were examined through multiplex PCR using seven different STS markers.
Results: Among 90 patients (45%) with positive micro TESE results 9 (10%) showed partial microdeletion in AZFc region. From 110 (55%) patients with negative micro TESE results 7 (6.3%) had AZC partial micro deletion. From 200 examined patients, 16 (8%) showed AZFc partial microdeletions including 11 (5.5%) gr/gr and 5 (2.5%) b2/b3. Statistical analysis showed no significant difference on micro TESE results between the patients with and without partial AZFc microdeletions.
Conclusion: Our findings do not confirm a positive relationship between presence of partial AZFc microdeletion (gr/gr or b2/b3) and micro TESE outcome in azoospermic men.
Keywords: Y Chromosome, AZFc, Partial Microdeletion, Micro TESE, Azoospermia

Ps-121: A Novel L-asparaginase Candidate for Acute Lymphoblastic Leukemia Treatment (ALL)

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Objective: Since 1970s, *E.coli* derived l-asparaginase has been used in the treatment of ALL. Some clinical trials report around 60% of patients produce antibody against this protein. These observations led to the search for alternative sources of l-asparaginase. This is the first report of cloning and characterization of a halophilic l-asparaginase which is expected to be more stable in blood, as a solution with different salts. The enzyme activity was measured in the presence of human serum. Specific activity was also determined since larger specific activity translates to less required amount of enzyme to achieve the same enzyme activity which in turn would reduce immunogenicity. Optimum temperature and pH was measured to determine whether the blood condition would be suitable for enzyme activity.
Materials and Methods: The gene sequence was obtained from ncbi database and amplified using PCR reaction. The PCR product was cloned in PET21 vector and transformed in E.Coli BL21.Purified enzyme (using nickel column) was used to measure optimum salt concentration and specific activity of *Halomonas Elongata* l-asparaginase.
Results: The enzyme preserve its activity in the presence of human serum . The specific activity of the enzyme was determined to be above 1500 IU per mg of protein which is amongst the highest reported specific activities for l-asparginases. Optimum temperature was 37 degree centigrade and maximum activity was retained in pH=6 to 9.
Conclusion: All evidence reflects that blood is a suitable medium for the activity of this enzyme. Halomonas Elongata l-asparaginase is expected to show high and stable activity in the Human blood (tempature 37 degree centigrade, pH=7.45, plasma water content of 90% (high solute concentration) and reactive to foreign protein (due to immune system)). All evidence reflects that blood is a suitable medium for the activity of this enzyme. Halomonas Elongata l-asparaginase is expected to show high and stable activity in the Human blood (tempature 37 degree centigrade, pH=7.45, and serum as a complex media for enzyme activity). Also, due to the high enzyme specific activity, lower dose of enzyme can be used to achieve therapeutic dose which mean lower activation of immune system.
Keywords: Leukemia, L-asparaginase, Halophilic Enzymes

Ps-122: Porous Scaffold for Bone Regeneration

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Objective: Bone defects due to trauma and to pathological and physiological bone resorption represent a major challenge and are a global health problem .A lot of research has been carried out to find out a substitute to support bone in the medical field. Bone tissue engineering is a very promising approach for the treatment of damaged bone and to overcome current clinical limitations. Recent efforts of bone repair focus on development of porous three dimensional scaffolds for cell adhesion and proliferation. collagen nanoHA cryogel scaffolds (70:30; 50:50; 30:70) were produced by cryogelation technique using EDC as crosslinking agents. A pure collagen scaffold was used as control. cell morphology (SEM), chemical analysis (FTIR) and porosity of scaffold was tested.
Materials and Methods: The collagen cryogel was prepared with 5 ml of collagen diluted in 4 ml of HCl (5mM) on ice bath. Subsequently, 20 mM EDC were added to the collagen slurry and transferred to a syringe (Terumo Syringe, 5ml) that was used as a mold. This was then kept in a freezer at -18°C for 24 hours to complete the crosslinking, then it was thawed at room temperature and the scaffold was washed with distilled water and finally dried with a freeze dryer. In the case of collagen-nanohydroxyapatite scaffolds, the dry powders of nanoHA aggregates were mixed with the HCl solution in a particular ratio (final composition collagen-nanoHA 70:30, 50:50 and 30:70 w/w %) and then cross linked as described above.
Results: SEM revealed that all cryogel scaffolds had highly porous structure with interconnective porosity and the na-

noHA aggregates were also randomly dispersed throughout the scaffold structure. FTIR showed the presence of all major peaks related to collagen and hydroxyapatite in the scaffolds and also indicated possible interaction between nanoHA aggregates and collagen molecules. Porosity analysis revealed an enhancement in the surface area as the nanoHA percentage increased in the collagen structure.
Conclusion: The results suggest that the presence of collagen-nanoHA biocomposite scaffolds resulted in higher overall cellular proliferation compared to pure collagen scaffold. A statistically significant difference between collagen and collagen-nanoHA cryogels was observed after 21 day of cell culture. Histological analysis of the cell-seeded scaffolds using hematoxylin-eosin staining revealed also that collagen scaffold presented lower cell density than biocomposite scaffolds. collagen-nanoHA cryogels could have potentially appealing application as scaffolds for bone regeneration.
Keywords: Porous Scaffold, Hydroxyapatite, Bone Regeneration

Ps-123: Cancer Stem Cell Biomarkers in The Diagnosis and Management of Liver Cancer

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Objective: In spite of recent therapeutic progress, liver cancer is the fifth prevalent cancer and the third factor for cancer-related mortality. Recently resistant liver cancer stem cells have been found to be responsible for tumor growth, metastasis and relapse. Targeting liver cancer stem cells is becoming the major strategy for evaluating the liver cancer treatment and novel therapeutic methods. These cells have some surface biomarkers such as CD133 and ALDH that can be used in diagnosis and management of patients.
Materials and Methods: In this study we analyzed the findings from articles in PubMed database. In this study by comparing different biomarkers in different articles, we are looking for results which make the future of liver cancer treatment clear.
Results: We found that these biomarkers can be used for the prediction of tumorigenesisand resistance to therapy in these patients. Tumorigenesity rate and cancer cell resistance increases in this order: $CD133^-$, $ALDH^-$>$CD133^-$, $ALDH^-$>$^+$CD133, $^+$ALDH. Furthermore, targeting these markers can sensitize tumor cells to chemotherapeutic agents. Without considering these biomarkers in the treatment, the disease may relapse. In routine treatment and without considering cancer stem cells, these cells would stay alive and cause disease relapse, but by considering these cells the treatment is possible and relapse will not happen.
Conclusion: Considering different liver cancer stem cell biomarkers helps to find new methods in therapy and also makes therapy more efficient. In future works nucleic acid biomarkers and their relation with liver cancer stem cells and their surface biomarkers may help to better treatment of liver cancer.
Keywords: Surface Biomarkers, Liver Cancer, Tumorigenesis, Stem Cells

Ps-124: Sophisticated Perusing of MYC and A miscellany of miRNAs in The Aggressive Types of Breast Cancer with Micrometastasis to Bone marrow

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Objective: In the genesis of breast cancer, diverse molecular mechanisms have been involved. Subsequently, different patterns of ectopic gene expression cooperate closely or away to agitate for implementing a latent metastasis to bone marrow. Furthermore, breast cancer cells who lodge in bone marrow should survive and grow in the new residence, so for this reason, they must quite subtly escape from the influence of TRAIL, CXCL12, factors of bone marrow metastasis microenvironment and etc.. In this ascertainment, we investigate the roles of C_MYC oncogene and miscellaneous arrays of miRNAs that could have complex collaborations with together for a stealth activity of breast cancer cells on this phenomenon. Indeed, MYC and privileged miRNAs clusters could modify the expression of genes underlying to perform micrometastasis simultaneous with growth of breast tumors whose are at different developmental stages.
Materials and Methods: We used miRNA prediction databases to predict miRNAs targeting 3´UTR of c-MYC mRNA and then by using of culturing aggressive breast cancer cell lines and recruiting tissue samples from patients that preferences of these tissue specimens for bone metastasis have been asserted and extracting total RNAs from the samples, we analyzed the expression levels of MYC and predicted miRNAs by precise primers and Real-time PCR using SYBR green (I).
Results: Evaluation of novel therapeutic targets is essential for strong clinical outcomes. Hereupon, with this ascertainment, we analyzed specific crosstalk between miRNAs and MYC from samples in osteolytic bone metastasis, as a result of our analysis, we found out miRNA_34a and miRNA_34c have an effective role on overexpression of MYC in BCC.
Conclusion: Taken together, in the invasion types of breast cancer, poorer clinical outcomes are the most indispensable result after breast tumor surgery and subsequent treatments in patients. Based on this assessment, we can provide the robust and novel therapeutic molecular targets with fewer recurrence consequences.
Keywords: MYC, microRNA, Metastasis, Bone Marrow, Breast Cancer TherapyI

Ps-125: Overexpression of Piwil2 Induces Cancer Stem

Cells Properties in MCF7 Breast Cancer Cell Line

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Objective: Piwil-like RNA-mediated gene silencing 2 (Piwil2) is a member of AGO/PIWI gene family which is enriched in cancer stem cells. Being highly expressed in different types of cancer, it can contribute to biological pathways such as the epithelial-to-mesenchymal transition (EMT) where the loss of cell-cell adhesion leads to a shift in the cytoskeletal dynamics of cells by changing the epithelial morphology of the cells to a mesenchymal phenotype. EMT is the main factor responsible for the progression of the cancer. The objective of this research was to investigate the overexpression of Piwil2 and its role in the induction of EMT and cancer stem cells properties in MCF7 breast cancer cell line.
Materials and Methods: MCF7 cells were transfected with a plasmid containing human Piwil2 that was under the control of CMV promoter utilizing Neon Electroporation method. Subsequently, selection was carried out using G418 and doubling time was calculated in the transformed and control cells. RT and real-time PCR were also performed to analyze the expression of epithelial and mesenchymal genes as well as those related to cancer stem cells.
Results: The molecular analyses revealed a significant reduction in the expression of epithelial genes (Ovol-2, E-cadherin and Zo-1) while a significant increase was detected in the expression of mesenchymal markers (Vimentin, Zeb-2, Slug, Snail and Fibronectin) as well as a few cancer stem cell biomarkers including Oct4, CD44 and ALDH.
Conclusion: Based on the results from this study it can be proposed that the ectopic expression of Piwil2 can trigger the process of EMT which loosens the tight extracellular connections of the cells rendering them invasive as they start to possess stem cell-like characteristics and adopt a mesenchymal morphology. Hence, Piwil2 protein seems to act as a master regulatory protein that is able to manipulate the transcription through specific signaling pathways which allow the cells to gain stem cell-like properties. Furthermore, it can participate in invasion and metastasis of tumor cells through activating the EMT process.
Keywords: Piwil2, Breast Cancer Stem Cells, Epithelial-to-Mesenchymal Transition, MCF7

Ps-126: Application of Human Umbilical Cord Mesenchymal Stem Cells in The Treatment of Duchenne Muscular Dystrophy

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Objective: Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy. Discarded umbilical cord (UC) is a potential source of mesenchymal stem cells which are non-immunogenic and can be used for transplantation in allogenic set ups. Given the regenerative and anti-inflammatory properties of mesenchymal stem cells (MSCs), here we investigated its role in the cellular therapy of DMD patients.
Materials and Methods: This is a single-blinded study conducted in Taleghani Hospital, Tehran, Iran. Boys aged between 7 to 21 years were enrolled in the study. UC-MSCs (2 million/kg body weight) were administered through IV and IM injection. Muscle power in muscles of proximal and distal upper limb, proximal and distal lower limb were measured in 11 DMD patients after UC-MSCs transplantation and were followed for up to 2 years (average follow up 1 years). 5 DMD patients did not receive any UC-MSCs transplantation and served as the control group.
Results: In the treatment group, the mean of strengths of proximal and distal upper limb remained stable after one year post-transplantation (N=9) compared to pre-transplantation (N=11) (3.78 vs. 3.45, $P>0.05$ in the proximal upper limb respectively and 4.22 vs. 4.09, $p>0.05$ in the distal upper limb respectively). In contrast, in the control group (N=5), the mean of strength of distal upper limb decreased after 1 year compared to baseline (3 vs. 4, $P<0.05$ respectively) while no statistically significant difference was observed in the proximal upper limb after 1 year compared to baseline (3.0 vs. 3.6, $P>0.05$ respectively). Similar results were observed in proximal and distal lower limb.
Conclusion: UC-MSCs administration not only resulted in the stabilization of muscle power but also did not show graft-versus-host disease (GVHD) or any deleterious effects on the patients and thus may be considered as safe option for treatment of DMD as compared to control untreated group.
Keywords: Duchenne Muscular Dystrophy, Umbilical Cord, Mesenchymal Stem Cells, Cell Therapy