ABSTRACT BOOK

ROYAN 2020

International Twin Congress

Reproductive Biomedicine & Stem Cells

September 2- 6, 2020 Tehran- Iran

21st September 2 - 4, 2020
Congress on
Reproductive Biomedicine

16th September 5 - 6, 2020
Congress on
Stem Cell Biology & Technology

Abstracts of

Royan International Virtual Twin Congress

16th Congress on Stem Cell Biology and Technology
5-6 September 2020

Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Abstracts of the 16th Congress on Stem Cell Biology and Technology (2020)

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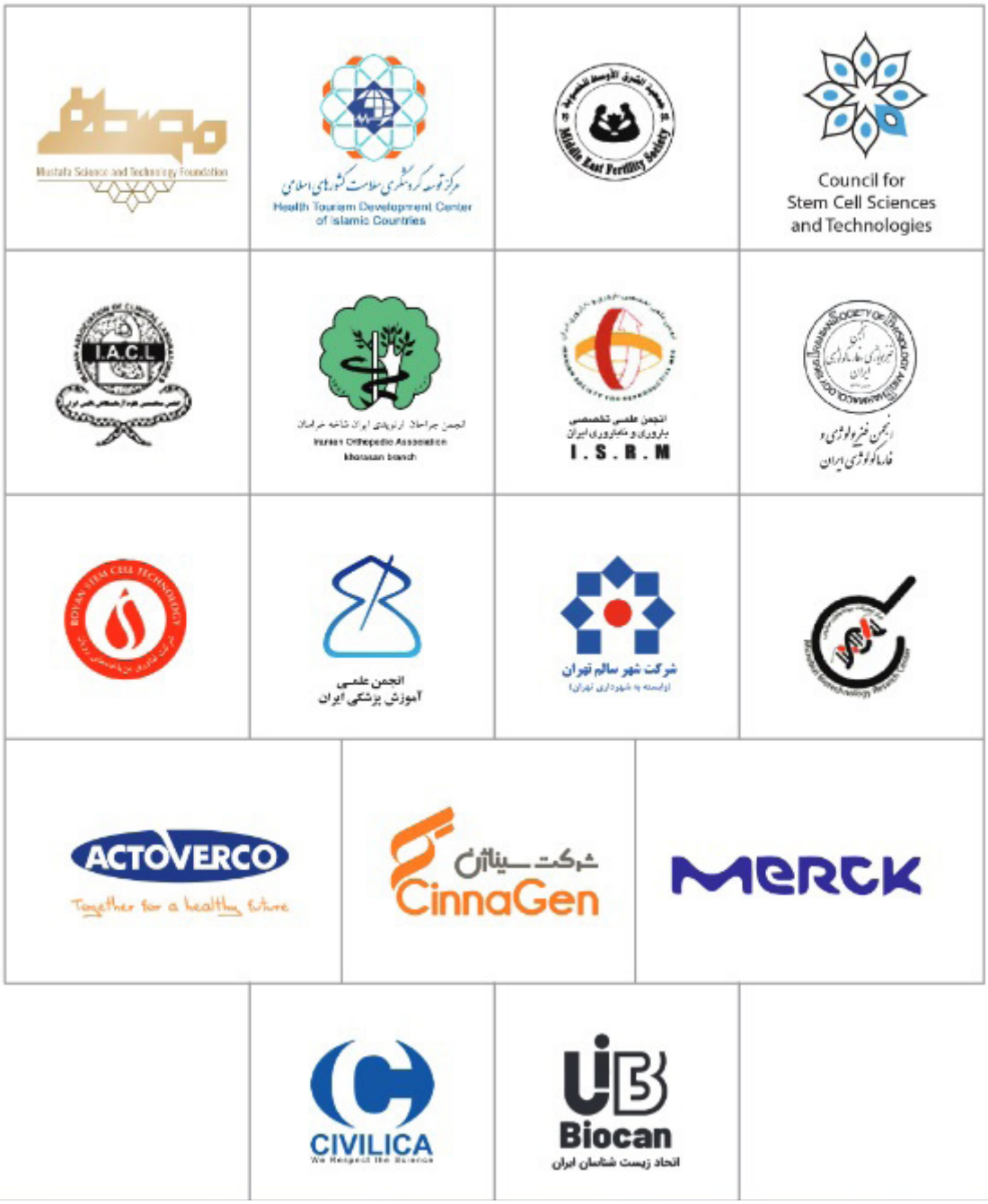
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Congress Chairperson

Leila Montazeri

Dear Colleagues,
On behalf of the organising committee, we are delighted to welcome you to the 16th Royan International Congress on Stem Cell Biology and Technology (ICSCBT) Virtual, which will be held on September 5-6, 2020. The congress is part of the 5-day virtual Royan International Twin Congress and is accompanied by 21st Congress on Reproductive Biomedicine.
The ICSCBT 2020 virtual features two days of the best in stem cells research and regenerative medicine covering different aspects of basic and translational contents. Due to the COVID19 pandemic across the world, we have decided to convert the congress to a fully virtual event.
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.
We warmly invite you to join us in the ICSCBT 2020 virtual to learn, share and network.

Kind regards
Leila Montazeri, PhD
Chairperson of 16th Royan International Congress
on Stem Cell Biology & Technology

Invited Speakers

Is-1: Stretch Induces Invasive Phenotypes in Breast Cells Due to Activation of Aerobic-Glycolysis-Related Pathways

Abdolahad M

School of Electrical and Computer Engineering, Tehran University, Tehran, Iran
Email: m.abdolahad@ut.ac.ir

It is increasingly being accepted that cells' physiological functions are substantially dependent to the mechanical characteristics of their surrounding tissue. This is mainly due to the key role of biomechanical forces on cells and their nucleus shapes which have capacity to regulate chromatin conformation and thus gene regulations. Therefore, it is reasonable to postulate that altering the biomechanical properties of a tissue may have capacity to change cell functions. Here, we probed the role of cell stretching (as a model of biomechanical variations) on cell migration and invasion capacity using human breast cells in both normal and cancerous conditions. Using several analyses (i.e., scratch assay, invasion to endothelial barrier, real-time RNA sequencing, confocal imaging and patch-clamp etc.) we revealed that cell-stretching process could increase the migration and invasion capabilities of normal and cancerous cells respectively. More specifically, we found that post stretched breast cancer cells had been found in low grades of invasion, substantially upregulate the expression of MnSOD through activation of H-Ras proteins which subsequently induces aerobic glycolysis (Warburg effect) followed by overproduction of matrix metalloproteinases (MMP) reinforced filopodias. Presence of such invadopodias facilitate the targeting of vascular endothelial layer and increased invasive behaviors in breast cells are observed.

Is-2: Adapting a 3D Microfluidic Culture System to Study Immune Checkpoint Blockade for Personalized Immunotherapy

Aref AR

Belfer Center for Applied Cancer Science, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA
Email: Amir_Aref@hms.harvard.edu

Is-3: Manufacturing New Neurons from Glia in Postnatal Brains

Berninger B

Institute of Psychiatry, Psychology and Neuroscience, Centre for Developmental Neurobiology, MRC Centre for Neurodevelopmental Disorders, King's College, London, UK
Email: benedikt.berninger@kcl.ac.uk

Direct lineage reprogramming emerges as an innovative strategy for cell-based therapy. In the postnatal and adult brain, different types of glial cells can be converted into neurons by forced expression of neurogenic transcription factors such as the proneural factors achaete-scute-like 1 (Ascl1) and neurogenin 2 (Neurog2). While there is ample proof-of-principle evidence for successful conversion, many aspects of the process remain enigmatic. In my presentation, I will discuss our efforts to understand the molecular and cellular mechanisms that underlie reprogramming into neurons and present our attempts at generating subtype-specific neurons in the mouse cerebral cortex *in vivo*.

Is-4: Exosomes as A Next-Generation Drug Delivery System: An Update on Drug Loading Approaches and Characterization

Haeri A

Department of Pharmaceutics and Pharmaceutical Nanotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: a_haeri@sbmu.ac.ir;

Exosomes are small nanoparticles secreted by almost all cells and have a well-known role in intercellular communication. They are found in different body fluids and can also be isolated from cell culture media. They contain a natural cargo including various protein and nucleic acid molecules originated from their donor cells. In recent years, exosomes provide a great potential as drug delivery and theranostic systems. They are believed to provide a targeted delivery of drug molecules, supplemented with their natural function. Furthermore, they have a membranous structure similar to liposomes, and that motivated researchers to apply their previous experience of drug loading into liposomes for exosomes. Herein, we discuss applied methods for the encapsulation of different drugs into exosomes, parameters affecting the incorporation of drug molecules into exosomes, characterization techniques, recent achievements, commercialization challenges and the potential future developments of exosomal drugs. Overall, while the application of exosomes as a drug delivery system is still in its infancy, they are considered to be a new class of natural nanocarriers with great potential for clinical application. Understanding of their key formulation parameters, pharmaceutical properties, *in vivo* behavior and applicable scale-up production will pave their way to the market.

Is-5: Novel Bone Morphogenetic Protein Delivery Systems for Skeletal Tissue Regeneration

Jabbari E

Department of Chemical and Biomedical Engineering, University of South Carolina, Columbia SC, USA
Email: jabbari@cec.sc.edu

Tissue morphogenesis during fetal development is highly dependent on spatial and temporal expression of multiple morphogens targeting different progenitor cells. During fetal development, mesenchymal stem cells (MSCs) and endothelial colony forming cells (ECFCs) are implicated in bone formation coupled by paracrine signaling between the two progenitor cells. The invading ECFCs secrete osteogenic morphogens (BMP2) to stimulate cell differentiation and mineralization whereas the differentiating MSCs release vasculogenic morphogens (VEGF) to further stimulate capillary formation for the metabolically-active osteoblasts. I will present in my seminar novel

nanomaterial-based strategies and tissue models for testing on-demand delivery of morphogens to progenitor cells in skeletal tissue regeneration.

Is-6: Stem Cell Engineering Approaches to Additive Bioassembly

Kilian K

School of Materials Science and Engineering, School of Chemistry, Australian Centre for Nanomedicine, University of New South Wales, Sydney NSW, Australia
Email: k.kilian@unsw.edu.au

The extracellular matrix surrounding cells in tissue is a dynamic composite material, where the presentation of biophysical and biochemical information directs functional bioactivities. My laboratory is interested in how the properties of the extracellular matrix guides cell decision making and have developed a suite of engineered extracellular matrices to probe the biophysical and biochemical basis underlying cell programming and re-programming. Here I will discuss how engineered model systems that better recapitulate *in vivo* biology than tissue culture plates can be leveraged to explore how cells receive and integrate signals to specify lineage. First, I will show how combinatorics can serve to identify hydrogel substrates that prime a therapeutic acitivity in mesenchymal stromal cells (MSC). MSCs adherent to extracellular specific matrix conjugated hydrogels show elevated secretion of pro-angiogenic and immunomodulatory cytokines and this activity is retained after cryopreservation and storage. Next, I will demonstrate a new bioprinting approach within a cell-laden bath of extracellular matrix colloids, where MSCs within the bath proliferate and differentiate when in proximity to bone-like structures. These and other composite hydrogel-based model systems are changing the way in which fundamental biological questions can be probed, which will aid our understanding of biological processes while revealing new design parameters for regenerative biomaterials.

Is-7: Future Trends in Bone Tissue Engineering

Khojasteh A

Department of Tissue Engineering and Applied Cell Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: arashkhojasteh@gmail.com

Despite all advances in bone tissue engineering (BTE), still autgogenous bone from intra oral or extra oral donor sites are the gold standard for treatment of large craniomaxilofacial defects. Biomaterial development, application of growth factor, and stem cells, open new gateway to bone regeneration studies, but real translation from bench to bedside have not yet happened. The actual invivo differentiation of stem cells, megadose application of growth factors, absence of proper vasculature in tissue engineered scaffolds are the main disadvantages of the current approaches for BTE. Changing cell sources, smart scaffolds, dynamic cell culture methods and bedside modification are the main approaches that scientists are trying to use to enhance success rate in bone regeneration.

Is-8: Cell-Free Regenerative Medicine for Neonatal Lung Disease

Lim R

1. Department of Obstetrics and Gynecology, Monash University, Hudson, Australia
2. Cell Therapy and Regenerative Medicine Platform, Hudson Institute of Medical Research, Hudson, Australia
Email: rebecca.lim@monash.edu

Is-9: Cell or Cell Products, which Would Rescue Cardiovascular Regenerative Medicine?

Pahlavan S

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sarah.pahlevan2007@gmail.com

Ischemic heart conditions are one of the top categories in the world health complications accounting for high mortality rates which estimated to be about 30% worldwide. Common therapeutic approaches including medications or device therapy have been widely used and been the option of choice for cardiologists resulting in fairly good outcomes. However, these approaches could not induce mascularization in the ischemic heart region and inhibition of progression into heart failure, possibly to its end stage which necessitates heart transplantation as the only remaining option. While heart transplantation is continued to be very challenging due to the shortage of organ donor. The 21st century has been beginning of a very promising era in the field of medicine as novel therapeutic approaches have been introduced, researched and found its way to the clinics particularly for diseases with no effective treatment. Cell therapy is one of these novel therapeutic approaches that attracted high attention and has been widely experimented for diseases with no efficient cure including ischemic heart conditions. Different modalities of cell therapy have been employed for ischemic heart diseases including multiple cell types, various dosing and delivery means. Although promising, the results of clinical trials seems to be confounded by inter-trial and inter-patient variability making an accurate conclusion challenging. And exactly for this reason, the recommendation of any cell type or dosing has been missing so far. Furthermore, although the results of mesenchymal stem cell therapy were promising for ischemic heart conditions, but still not relevant enough to be translated and be routinely used in clinics. In parallel to investigate cell therapy, interest in cell products' therapy of myocardial infarction (MI) has followed due to the potential of such modalities for clinical translation, high replicability and off-the-shelf accessibility. One of these promising approaches is transplantation of extracellular vesicles (EVs) to infarcted heart tissues. EVs include microvesicles (MVs), apoptotic bodies and exosomes. It has been shown that therapeutic efficacy of cardiac cell therapy was to a great extent due to EVs. EVs carry functional proteins and various types of RNA that can inhibit remodeling in the infarcted myocardium. In general, the insufficient efficacy of cell or cell products therapy so far has been mainly ascribed to limited potencies of these novel therapeutic agents, limited retention after delivery, and disease heterogeneity. Thus, ongoing efforts are made to achieve to the best setting including best cell source with maximal biotherapeutic potency or best cell source products, standardized dosing, the most feasible and efficient delivery system, and last but not least the appropriate target responsive patient populations.

Is-10: Epigenome Editing Technology, A Promising Tool in

Neuroscience Studies

Pahlevan M
Department of Clinical Neuroscience, Neuroimmunology Unit, Center for Molecular Medicine CMM, Karolinska Institute, Solna, Sweden
Email: majid.pahlevan.kakhki@ki.se

Is-11: Regeneration of Spinal Cord Connectivity through Stem Cell Transplantation, Biomaterial Scaffolds, and Pharmacological Approaches: Human Randomized Clinical Trial; Who? When? What? How?

Rahimi Movaghar V

1. Spine Program, University of Toronto, Toronto, Canada
2. Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, Iran
Email: v_rahimi@yahoo.com

Is-12: An Antibody Against Early Driver of Neurodegeneration Common among Alzheimer's, Bipolar and Diabetes

Shahpasand K[1], Farhadi A[1], Tahmtani Y[1], Zhou X[2], Lu KP[2]

1. Department of Brain and Cognitive Sciences, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
2. Division of Translational Therapeutics, Department of Medicine, Cancer Research, Institute, Harvard Medical School, Boston, MA, USA
Email: shahpasand09@gmail.com

Objectives: Traumatic brain injury (TBI) is one of the major risk factor for Alzheimer's disease (AD), whose pathological hallmarks include tau hyperphosphorylation. However, so far tauopathy has been undetectable acutely after TBI and how TBI leads to tauopathy which in turn would increase risk of AD remains unknown. We herein identify a neurotoxic cis conformation of phosphorylated tau at Thr231, as a major early driver of tauopathy and neurodegeneration, which is common among several tauopathy disorders, that is effectively blocked by the conformation specific monoclonalantibody.
Materials and Methods: We immunostained control and human TBI, AD and Bipolar brains with our cis/trans monoclonal antibodies. Also, we examined TBI mouse models of Diabetes and Macular degeneration, treated with either control IgG or cis mAb employing immunostaining and electron microscopy. Moreover, we studied risk-taking behaviour of those TBI mice.
Results: We found robust cis p-tau in those TBI, AD, BD human postmortem brains. Acutely after TBI in mice, neurons prominently produce cis p-tau, which disrupts axonal microtubule network, spreads to other neurons, and leads to apoptosis, a pathogenic process, which we termed "cistauosis" that appears long before known tauopathy. Also, while TBI causes abnormal risk-taking behaviour in mouse models, cis antibody treatment restores thephenomena.
Conclusions: Treating TBI mice with cis antibody prevents tauopathy development and spread, and restores brain histopathological and functional outcomes. These results uncover cistausosis as an early driver of tauopathy and neurodegeneration upon TBI. We anticipate that cis p-tau will be a new early biomarker and that cis p-tau antibody may be used to treat or even prevent TBI, chronic traumatic encephalopathy and AD.

Is-13: Connected Mobile Health Diagnostics with Applications in Infertility Management

Shafiee H

Division of Engineering in Medicine and Renal Division of Medicine at Brigham and Women's Hospital,Harvard Medical School, USA
Email: hshafiee@bwh.harvard.edu

The advances in micro- and nano-technologies and the surge in consumer electronics have paved a solid foundation for developing mobile health (mhealth) technologies with the potential to transform the current paradigm in global health. Dr. Shafiee's laboratory strives to develop mhealth diagnostics and solve unmet medical problems through bioengineering approaches and utilizing the advances in consumer electronics such as cellphones, nanoscale/microscale materials, and computer programing particularly artificial intelligence (AI). mhealth technologies offer novel approaches to diagnose, track, and control diseases including infectious diseases, cancer, and infertility in both resource-rich and resource-poor settings. In this talk, Dr. Shafiee will present examples of how smartphones can be seamlessly integrated with hardware, software, microfluidics, and nanotechnology to develop point-of-care diagnostic devices to address clinical gaps in the management of infertility and infectious diseases.

Is-14: Characterizations of Molecularly Distinct Subpopulations of Evs: *In Vitro* and *In Vivo* Studies

Shekari F

Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: faezehshekari@gmail.com

Recently, the possibility of replacing cells or liposomes with the naturally secreted micro-and nano scale extracellular vesicles (EVs) has sparked a heated debate. With its capacity to be used as therapeutics agent, cell conditioned medium derived EVs (CM-EVs) play an explosive role in the realm of regenerative medicine or pharmaceutical science. Increasing and compelling evidence additionally render EVs potentially promising options to be used as "commercial off-the-shelf product". Accepting the heterogeneity in EV subpopulations isolated by different methods, in this report, following the isolation of EVs by different methods (ultracentrifuge, sucrose gradient, and 20,000 g centrifugation only), we confirmed that all of them met MISEV2018 criteria (size, morphology, and presence of 3 positive markers and absence of 1 negative marker). Further assessments has been done to find out the purity (based on albumin contaminant), vesicular membrane perfectness (phosphatidylserine exposure), as well as their functions in in-vitro tests (cellular uptake and proliferation), *ex vivo* test of angiogenesis and *in vivo* administration to monkey model of diabetes. Large scale proteome analysis of EVs sub-populations has also provided us with some important clues to choose between tests and functions. In conclusion, we demonstrate that molecularly distinct EV subpopulations can be evaluated by quantifiable methods. Further assessments to correlate some functions to each subpopulation prior to designing clinical trials can accelerate the development of EV-based therapeutics.

Is-15: Epithelial Mesenchymal Transition in Carcinoma; Therapeutic Intervention

Thiery JP

1. Guangzhou Regenerative Medicine and Health, People's Republic of China
2. Research Director emeritus, Comprehensive Cancer Center Institute Gustave Roussy, Villejuif, France
Email: tjp@nus.edu.sg

We previously proposed that epithelial-mesenchymal transition (EMT), a developmental mechanism, becomes high-jacked by carcinoma cells for their invasion and metastasis and have established an EMT scoring system applicable to all carcinoma. In this presentation, I will briefly discus when and how an EMT phenotype can be acquired in the primary tumors or in CTCs. I will then describe our strategy to harness the EMT concept as a complementary strategy to currently available targeted therapeutics. I shall present the potential to treat mesenchymal-like tumors in bladder carcinoma based on our analysis of the mechanisms driving EMT in this tumor type. I will also show that we can potentially improve immunotherapy-based treatment by interfering with the EMT phenotype.

Is-16: Deepathology: Deep Multi-Task Learning for Inferring Molecular Pathology from Cancer Transcriptome

Sharifi-Zarchi A

Department of Computer Engineering, Sharif University of Technology, Tehran, Iran
Email: asharifiz@gmail.com

Is-17: Innovative Niche for Stem Cell Research

Tahamtani Y

1. Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Diabetes, Obesity and Metabolism, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: yasertahamtani@royaninstitute.org

Poster Presentations

Ps-1: Targeting The PI3K/AKT, Wnt/B-Catenin and Tgfβ Pathways by Small Molecules in Hematopoietic Stem Cell Expansion

Afzal E[1*], Zarrabi M[1, 2], Asghari MH[2], Ebrahimi M[2]

1. Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: marzieh.ebrahimi@gmail.com

Objective: Small molecule compounds have been well recognized for their promising power in generation, expansion and maintenance of embryonic or adult stem cells. The aim of this study was to identify a novel combination of small molecules in order to optimize the *ex vivo* expansion of umbilical cord blood derived-CD34+ cells.
Materials and Methods: Considering the most important signaling pathways involved in the self-renewal of hematopoietic stem cells, cord blood derived-CD34+ cells were expanded with cytokines in the presence of seven small molecules including SB, PD, Chir, Bpv, Pur, Pμ and NAM. Eliminativism approach was used to find the best combination of selected small molecules for effective *ex vivo* expansion of CD34+ cell. In each step, proliferation, self-renewal, and clonogenic potential of the expanded cells as well as expression of some hematopoietic stem cell related genes were studied. Finally, the engraftment potential of expanded cells was also examined by the mouse intra-uterine transplantation model.
Results: Our data shows that simultaneous use of SB431542 (TGF-β inhibitor), Chir9901 (GSK3 inhibitor) and Bpv (PTEN inhibitor), resulted in a 50-fold increase in the number of CD34+CD38- cells. This was further reflected in approximately 3 times increase in clonogenic potential of the small molecule cocktail-expanded cells. These cells, also, showed a 1.5-fold higher engraftment potential in the peripheral blood of NMRI model of in utero transplantation. These results are in total conformity with up-regulation of HOXB4, GATA2 and CD34 marker gene as well as CXCR4 homing gene.
Conclusion: Taken together, our findings introduce a novel combination of small molecules to improve the yield of existing protocols used in the expansion of hematopoietic stem cells.
Keywords: Cord Blood, Hematopoietic Stem Cells, Small Molecules, *Ex Vivo* Expansion

Ps-2: Hypertrophic Gene Expression in An *In Vitro* Pathological Model of XX and XY Human Embryonic Stem Cells (hESCs)-Derived Cardiomyocytes

Ahmadvand Sh, Meyfour A, Pahlavan S

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: shiva.ahmadvand@gmail.com

Objective: Gender-specific phenotypes of the cardiovascular system have been reported with respect to both physiology and pathology. While most of these sex-related differences have been associated with the sex hormones, molecular biology studies have assigned roles to the differential expression of genes from which many are located on X-Y chromosomes. Therefore, in the present study, we sought to investigate the expression of the cardiac-specific genes in samples provided by cardiogenic differentiation of XX and XY human embryonic stem cells (hESCs) during different stages of differentiation in hormone-free setups.
Materials and Methods: We sought to investigate the expression of hypertrophic genes in XX and XY hESC-derived cardiomyocytes subjected to *in vitro* hypetrophy induction. Quantitative Real-Time PCR was used for gene expression analysis.
Results: The sex-related RPS4X gene showed down-regulation in males at days 6 and 12 of cardiac differentiation. The results of *in vitro* hypertrophy induction indicated that female cardiomyocytes were less sensitive to pathological stimulation because hypertrophy induction was achieved with higher doses of hypertrophy stimulant; isoproterenol. The ratio of NPPB/NPPA gene expression was significantly higher in hypertrophied male cardiomyocytes indicating a more relevant response. Interestingly, the expression of KDM5C of X chromosome was up-regulated in hypertrophied male cardiomyocytes.
Conclusion: In summary, our results may provide evidences on the direct contribution of differential gene expression on sexual dimorphism observed in physiological and pathological characteristics of cardiomyocytes.
Keywords: Embryonic Stem Cells, Cardiomyocyte Differentiation, Sexual Dimorphism, Hypertrophy

Ps-3: Study of A Novel Nanofiber-Containing Ring- Implanted Contact Lens for Corneal Sustained Delivery of Hyaluronic Acid

Akbari E[1*], Imani R[1], Shokrollahi P[2], Heidari keshsel S[3]

1. Department of Biomedical Engineering, Amirkabir University of Technology (Tehran polytechnic), Tehran, Iran
2. Department of Biomaterials, Faculty of Science, Iran Polymer and Petrochemical Institute, Tehran, Iran
3. Department of Tissue Engineering and Applied Cell Science, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: r.imani@aut.ac.ir

Objective: Dry eye syndrome, as a kind of persist corneal epithelial defect (PED), is an inconvenient ocular disorder that is generally treated by high-dosage, conventional eye drops. Addressing low efficacy, and bioavailability of conventional eye drops, drug-containing contact lenses are widely used as alternatives for eye drops in ophthalmic drug delivery applications.
Materials and Methods: In the present study, a novel nanofiber-containing ring implant poly(vinyl alcohol) (PVA) hydrogel is used as a carrier for hyaluronic acid (HA) delivery. HA is physically encapsulated in a nanofiber-containing ring-shaped hydrogel with a 2 mm width that is implanted in the final contact lens hydrogel.
Results: The designed contact lens has 59% porosity, 275% swelling ration, more than 95% transparency, and no weight loss in physiological condition. *In vitro* release studies are performed with implanted contact lenses with and without nanofibers. Nanofiber incorporation in the designed contact lens

was highly effective in decreasing burst release and sustaining the release over 14 days. In addition, nanofiber incorporation in the designed contact lens not only circumvent the changes in optical and physical properties, such as transparency, of the hydrogel, also strengthen its physical stability and increased the young modulus of the PVA hydrogel from 6 to 10 kPa. Cellular studies also revealed no cell cytotoxicity and cell attachment.
Conclusion: The study demonstrated the effective role of nanofiber in strengthening contact lens's physical stability, and also the promising potential of the designed contact lens to deliver HA for an extended period of time.
Keywords: Dry Eye, Hyaluronic Acid, Contact Lens, Sustain Release, Nanofibers

Ps-4: The Effect of Serum from Sepsis Patients on The Expression of CXCR7 Gene in Human Umbilical Cord Blood Mesenchymal Stem Cells *In Vitro*

Ameri F[1*], Haji Qasem kashani M[1], Atashi A[2], Bitaraf FS[3]

1. Department of Cellular and Molecular Biology, School of Biology and Institute of Biological Sciences, Damghan University, Damghan, Iran
2. Department of Medical Sciences, Faculty of Paramedical Sciences, Shahroud University, Shahroud, Iran
3. Department of Biotechnology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
Email: ameri.f458@yahoo.com

Objective: Sepsis is a common cause of mortality in patients admitted to intensive care unit of hospitals. In advanced stage, the disease leads to the death of patients with dysfunction of vital organs such as the heart, kidneys and liver. Mesenchymal stem cells (MSCs) enhance the regenerative capacity of damaged tissues by migration and homing in these tissues. The CXCR7 gene plays an important role in the homing and persistence of MSCs in the target tissues.
Materials and Methods: For this purpose, the serum of 20 patients with sepsis was collected. Human Umbilical Cord Blood Mesenchymal Stem Cells (hUCB-MSCs) were cultured in plate, four groups were treated with patient's serum and control group treated with serum from healthy volunteers. After 24 and 48 h, stem cells were trypsinized and extracted RNAs. cDNA was synthesized using reverse transcription reaction and special kit.
Results: The expression rate of this gene was determined by quantitative Real-Time PCR. The results showed that the expression of CXCR7 gene in hUCB-MSCs treated with patient's serums was increased significantly compared to the control group after 24 h. Also, there was a significant increase of this gene expression in 48 h treatment compared to the control and 24 h treatment.
Conclusion: High expression of CXCR7 gene improved efficiency of cell therapy to repair damaged tissues caused by sepsis.
Keywords: Sepsis, CXCR7 Gene, Human Umbilical Cord Blood Mesenchymal Stem Cells, *In Vitro* Culture

Ps-5: Expression of Runx-2 and Ocn Genes in Rat Bone Marrow Stem Cells Post-Induced by Osteogenesis Differentiation Medium and Electromagnetic Field

Amirahmadi F*, Kashani M, Nasiri M

Department of Cellular and Molecular Biology, School of Biology and Institute of Biological Sciences, Damghan University, Damghan, Iran
Email: fateme.amirahmadi11@gmail.com

Objective: Bone marrow stem cells (BMSCs) are one of the most important sources for repairing bone lesions in tissue engineering. The effect of sinusoidal electromagnetic field on the expression of osteogenic genes has been reported.
Materials and Methods: In this study, BMSCs of rats after confirmation by flow cytometry, were evaluated in three experimental groups: negative control (cultured cells in α-MEM, 10% fetal bovine), positive control (cultured cells in osteogenesis differentiation medium), and OD+50 (exposed cells to electromagnetic field with a frequency of 50 Hz, intensity of 1 mT daily for half an hour and osteogenesis differentiation medium). After 14 days, the extracellular matrix was stained with Alizarin Red and expression of Runx-2 and Ocn genes was assessed by quantitative Real-Time PCR, 14 and 21 days post induction. Differentiation into adipogenic and osteogenic cells was observed with Oil Red-O and Alizarin Red staining.
Results: The regions that responded positively to the staining were higher in the OD+50 group than in the positive control. On days 14 and 21, the expression of Runx-2 and Ocn genes in OD+50 showed a significant increase compared to positive control. While the intergroup comparison showed that the expression of these genes in the OD+50 group on day 21 was not significantly different from those of day 14.
Conclusion: As a result, the sinusoidal electromagnetic field had a stimulatory effect on the mRNA levels of Runx2 and Ocn genes expression in BMSCs treated with the electromagnetic field and the osteogenesis differentiation medium simultaneously. The electromagnetic field accelerates fracture healing by stimulating osteogenesis.
Keywords: Bone Marrow Stem Cells, Electromagnetic Field, Runx-2 and Ocn Genes

Ps-6: Simultaneous Treatment of Photobiomodulation and A Hybrid Graft of Seeded Human Demineralized Bone Matrix with Adipose-Derived Stem Cells Improve Osteogenesis in An Experimental Model of Critical-Sized Osteoporotic Defect

Asgari M[1*], Gazor R[2], Abdollahifar MA[1], Kiani P[3], Zare F[1], Amini A[1], Khosravipour A[1], Kazemi M[1], Chien S[4], Bayat M[1, 4]

1. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Anatomy, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
3. Department of Neuroscience and Addiction Studies, School of Advanced 37 Technologies in Medicine, Tehran University of Medical Science, Tehran, Iran
4. University of Louisville, and Noveratech LLC, Louisville, Price Institute of Surgical Research, Kentucky, USA
Email: bayat_m@yahoo.com

Objective: This research aimed to test the impact of simultaneous treatment of Photobiomodulation (PBM) and hybrid BTE scaffold on accelerating the healing rate of a critical-size femoral defect (CSFD) in an osteoporotic rat model.
Materials and Methods: The rats with CSFD were arbitrary separated into 6 groups: control, Scaffold (S, DBM), S + PBM, S + alendronate (ALN), S + ASCs, and S + PBM + ASCs. Each group was assessed by Cone Beam Computed Tomography

(CBCT) and histological examinations.
Results: At the 4th week, the largest volume of new bone formed in the S + PBM and S + PBM + ASC groups. S + PBM treatment relative to S and S + ALN treatments remarkably reduced the defect area. S + PBM + ASCs treatment compared to the S and S + ALN treatments significantly decreased defect area. At the 8th week, the defect area in the S + PBM group was substantially smaller than S, S + ALN and S + ASCs groups. Histological observations showed more new bone formation in the treated defects of S + PBM + ASCs and S + PBM groups.
Conclusion: We conclude that the simultaneous treatment of osteoporotic rats with PBM plus DBM with or without seeded ASCs enhances bone healing.
Keywords: Photobiomodulation, Demineralized Bone Matrix, Human Adipose-Derived Stem Cells

Ps-7: A Novel Method for Extracting Testicular Tissue Extracellular Matrix

Bashiri Z[1, 2*], Amiri I[3], Gholipour Malekabadi M[2, 4], Falak R[5], Asgari HR[1, 2], Koruji M[1, 2]

1. Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Department of Anatomical Sciences, School of Medicine, Hamadan University of Medical Sciences, Tehran, Iran
4. Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
5. Immunology Research Center (IRC), Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
Email: koruji.m@iums.ac.ir

Objective: The development of the decellularized extracellular matrix (ECM) scaffolds is able to open a new perspective towards regenerative medicine approaches. The use of transplanted testicular (TT) scaffolds with support of testicular cells may be an option for maintaining fertility in patients. Therefore, the purpose of this study was to prepare a decellularized testicular tissue as a suitable biomaterial for spermatogonial cell culture.
Materials and Methods: Fragments of testicular tissues were decellularized using three protocols: NaCl buffer, NaCl buffer-Triton, and sodium dodecyl sulfate (SDS)-Triton (ST). In all groups, urea buffer was used for solubilization of testis ECM. After centrifuge, the supernatant was removed and dialyzed. Finally, the contents of the dialysis tubes were centrifuged to remove polymerized proteins, and the supernatant (viscous matrix) was collected. The removal of the cells from tissues was confirmed by DAPI and Hematoxylin and eosin (HandE) staining, and DNA content assay. Alcian blue, Orcein and Masson's trichrome (MT) staining were used to confirm that ECM remained intact.
Results: According to staining results, all protocols were able to completely decellularized testis tissue. Furthermore, no residual nuclei were observed by DAPI and HandE staining. In NaCl buffer and NaCl-Triton groups, elastic, collagen fibers, and glycosaminoglycans were kept in decellularized testis tissue. But the structure of ECM was damaged in the ST group. In the NaCl group, DNA levels were significantly higher than other groups ($P<0.05$).
Conclusion: As a result, our study showed a valid and high-performance protocol for testicular testis decellularization. The use of decellularized TT can be useful for testicular tissue engineering purposes.
Keywords: Extracellular Matrix, Testicular Tissue, Decellularization

Ps-8: Evaluation of Silk Fibroin Nanofibrous Dressing Incorporating Niosomal Rosemary, for Potential Use in Wound Healing

Behyari M*, Imani R, Keshvari H

Department of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran
Email: r.imani@aut.ac.ir

Objective: Among wound care products, nanofibrous structure incorporating bioactive ingredients with the potential of sustained and localized release are currently attracting much more attention. Selecting proper bioactive agents, optimizing nanofibers structure/ characteristics as well as tuning the agent loading/ releasing plays a key role in the therapeutic effectiveness of such products.
Materials and Methods: Here, niosomal nanocarrier loaded with the REO is synthesized by the lipid film hydration technique. Morphology and sizes were evaluated by by Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS), respectively. Nano-niosomal formulation of effective herbal ingredient, Rosemary Essential Oil (REO) is introduced in silk (SF)-based nanofibrous mat via blend electrospinning and evaluated as a wound dressing. REO release from nanofibrous dressing was calculated with the uv-visible method. The viability and morphology of L929 fibroblast cells were evaluated on drug-loaded niosomal nanofibrous dressing, and the antimicrobial activities of the prepared samples were assessed by the agar diffusion method against S. aureus and E.coli.
Results: According to results, size and encapsulation efficacy of drug-loaded niosomes are 351.870 nm, and $68.51 \pm 0.762\%$, respectively, and the niosomes were successfully dispersed in the SF nanofibers, as confirmed by fluorescent microscopy. Embedding niosomal drugs into the SF nanofiber remarkably retarded the fast releasing of drugs which are nakedly embedded in the nanofibers where for REO, sustained-release with the almost same pattern are observed during 14 days of assessment. Cell culture study confirms that the drug-loaded niosomal nanofibrous dressing could promote greater cell growth and proliferation of cells after 72 hours when compared to the other dressings. Niosomal REO (10%) - incorporated nanofibers, as the optimized formulation, provides proper antibacterial activity against both S. aureus and E.coli.
Conclusion: Considering the results, it could be concluded that the incorporation of niosomal REO in the SF nanofibers with the potential of sustained-release seems a good candidate as bioactive and antibacterial wound dressing suitable for wound healing applications.
Keywords: Wound Dressings, Nanofibers, Niosomes, Herbal Ingredients, Controlled Release

Ps-9: Study of Gene Expression Profile in Non-Invasive and Invasive Retinoblastoma

Bereimipour A[1*], Miri MS[1], Karimi S[2], Hedayati Asl AA[3], Taleahmad S[4], Satarian L[1]

1. Department of Brain and Cognitive Sciences, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Ophthalmology, Ophthalmic Research Center, Torfe Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: lsatarian@gmail.com

Objective: Retinoblastoma (RB) is a malignant intraocular cancer of childhood with around 9,000 new cases a year. There are two kinds of RB: non-invasive and invasive, generally the later goes to morbidity and mortality due to secondary tumors. Family history in RB1 gene mutation and paternal age play a significant role in RB invasion. RB late diagnosis cause to more enucleation in developing countries. The discovery of biomarkers and important signaling pathways for discrimination between invasive and non-invasive phases is necessary. In this study, by using the bioinformatics approach, we investigated the gene expression and microRNA profiling to find the differentiating biomarkers concerning non-invasive and invasive phases.
Materials and Methods: We have specified the differentially expressed genes (DEGs) extracted from microarray libraries of non-invasive and invasive RB (GSE97508). Also, two miRNAs datasets in serum (GSE41321) and tissue (GSE7072) from GEO database and in-silico analysis were performed using enrichment databases such as EnrichR, KEGG, Panther, TargetScan and STRING on signaling pathway, gene ontology, and protein and miRNAs networks.
Results: Our results show that, 184 genes were significantly downregulated and 127 genes upregulated in invasive group compared to non-invasive. NTNG1 and COL4A1 were selected as upregulated genes due to their presence in extracellular matrix and GUCA1C and ARR3 as downregulated genes. Among the expression profile of miRNAs in serum and tissues, miR-20a and -204, show the more accurate evaluations because they both are involved in MAPK, VEGF, TNF, Ras signaling and Cell adhesion molecules Which are associated with invasion retinoblastoma
Conclusion: Using comprehensive bioinformatics analyzes, we could identify some genes and miRNAs as biomarkers in invasive and non-invasive retinoblastoma. Selected miRNAs by this study can be considered as biomarkers for improving the early detection of retinoblastoma.
Keywords: Retinoblastoma, Non-Invasive, Invasive, Biomarker, miRNAs

Ps-10: Finding Histone Modification Enzyme in Heart Development after Reprogramming of Fibroblast into Cardiomyocyte

Falsafi N*, Fallahi H

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: h.fallahi@razi.ac.ir

Objective: Cardiac cells have limited proliferation capacity and transplantation is a possibility that it is not always a good strategy, due to risk of tumor formation, so it is highly in demand to directly reprogram fibroblasts to cardiomyocyte. Some previous reports showed that fibroblast can be converted to the cardiac muscle by either a combination of transcription factors or the addition of miRNAs. Moreover, recent research implements a set of chemicals to successfully convert fibroblast to cardiac. This conversion includes opening chromatin structure by histone-modifying enzymes to express cardiac-specific genes. In thishis study, we identified histone modification enzyme which should be expressed during development of the heart. To find this, we utilized an online database from direct conversion by TFs and Chemicals in a few days after reprogramming (6 datasets and 1 dataset, respectively).
Materials and Methods: First, differentially expressed genes were extracted from NCBI using GEO2R online program. Next, histone modification enzymes (methylation, acetylation, phosphorylation, ubiquitination) and heart development enzymes were extracted from AmiGO to have experimentally validated gene names. Then, genes which are expressed with a fold change above 1 and p value less than 0.05 were identified and compared with genes list from Amigo. Finally, we obtained a number of validated DEGs related to histone modification during the early stage of reprogramming to cardiac muscles.
Results: Clustering of DEGs showed that most of them were related to histone acetylation and methylation. Only one gene was found as a histone phosphorylation enzyme and there was no histone ubiquitination enzyme among them.
Conclusion: These findings would be helpful to illustrate key histone modification enzymes which help to continue reprogramming by expression of cardiac development genes. Moreover, generating functional heart cells can maintain their features after induction. Hence, these cells would be replaceable by non-functional cardiac muscles without losing their capacity to remain stable.
Keywords: Heart Development, Histone Modification, Gene Expression

Ps-11: A Systems Biology Approach to Gene Expression Analysis between Triple Negative Breast Cancer and Metastatic Hepatocellular Carcinoma

Ghalavand M[1, 2*], Mohammadi- Yeganeh S[2, 3], Dorostkar R[2], Hashemi SM[4]

1. Department of Biotechnology, School of Advanced Technology in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Applied Virology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: s.mohammadiyeganeh@sbmu.ac.ir

Objective: Triple negative breast cancer (TNBC) is a type of breast cancer in which estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER-2) are not expressed. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults, and is the most common cause of death in people with cirrhosis. Cancer from other organs also may spread to the liver. Other common sites are the lung, and brain. In this study we find the important and common

genes that are therapeutic targets in these two diseases.
Materials and Methods: Genes related to TNBC and HCC were extracted from Genecard database. Then Common genes of these two diseases were identified by venn diagram. The network was constructed by Cytoscape software (version 3.5.1). Then main component of the network was analyzed considering centrality parameters including degree, betweenness, closeness and stress. Furthermore, Gene Ontology (GO) analysis of the key genes was performed.
Results: Output of genecards database shown 1550 genes in TNBC and 22399 genes in HCC are differentially expressed. 875 genes were common between TNBC and HCC. TP53, HRAS, MYC, PTEN, KRAS, CCND1, EGFR, CDKN2A, ESR1, ERBB2, AKT1, VEGFA, STAT3, NOTCH1, CTNNB1, BRCA1, JUN, EGF are the genes with high degree among the genes respectively. 14 crucial genes were analyzed by GO analysis, among all, molecular function "protein kinase binding" (GO:0019901), was disclosed as top category followed by Cellular Component "nuclear chromatin" (GO:0000790).
Conclusion: The analyses of common genes of the TNBC and lung cancer showed that there are some common crucial genes including TP53, HRAS, MYC, PTEN, KRAS, CCND1, EGFR, CDKN2A, ESR1, ERBB2, AKT1, VEGFA, STAT3, NOTCH1, CTNNB1, BRCA1, JUN and EGF which are tightly related to progress of disease. Therefore, these genes and pathways can be considered as an appropriate target for control and treatment of TNBC and and HCC.
Keywords: Hepatocellular Carcinoma, TNBC, Cytoscape, Gene Ontology

Ps-12: Histological Study of The Effect of Chitosan Scaffolds Loaded on The Different Concentrations of Hesperidin Flavonoid in The Rat Model of Femoral Fracture

Gharibblouk M*, Kashani M, Salehi M

Department of Cellular and Molecular Biology, School of Biology and Institute of Biological Sciences, Damghan University, Damghan, Iran
Email: jalalian.king71@gmail.com

Objective: Despite the success of bone graft in the healing of fracture, it has several limitations relating to donor-site morbidity, infection, bleeding, nerve damage and chronic pain. For that, the alternative biomaterials as scaffolds should be made with major influences on cell adhesion, proliferation and differentiation. Hesperidin, citrus flavonoid, inhibits bone loss by decreasing osteoclast formation and function. In this research, chitosan scaffolds with different concentrations of Hesperidin in rat femur defect repair were investigated.
Materials and Methods: Thirty adult male Wistar rats weighing 200-250 g were used and after creating fracture line on the right femur, they were divided randomly into six groups: control (femoral fracture model), 5 groups treated with chitosan scaffold with different concentrations of Hesperidin (0, 0.01, 0.1, 1 and 10%). The chitosan scaffolds were prepared from solutions with chitosan (at concentration of 1200mg/60cc acetic acid1%) and evaluated wettability, porosity, degradation and compatibility by MTT, SEM and FTIR. After 4 month, animals were sacrificed and the lesiond femurs extracted and stained with Hematoxylin and eosin (HandE) and Masson's trichrome (MT).
Results: The results of MTT showed that the scaffold has no toxic effects on stromal cells. SEM and FTIR results showed the improvement of cell-scaffold interactions. Histological and CT-Scan studies showed that the defect in treated groups (1 and 10%) was fully replaced by new bone and connective tissue compared to control and Hesperidin (0,0.01, 0.1%) treated groups. A stable collagenous matrix suitable for the osteoblastic proliferation and differentiation was formed in Hesperidin 10% treated group compared to control.
Conclusion: It seems that the chitosan scaffold loaded with 1 and 10% of Hesperidin has a potential role in the healing process bone lesions and it can be suitable as a therapeutic strategy in tissue engineering.
Keywords: Tissue Engineering, Scaffold, Chitosan, Hesperidin, Fracture

Ps-13: Human Fetal Pancreas Mesenchyme for Maturation of Human Embryonic Stem Cell Derived Pancreatic Organoids

Ghezelayagh Z[1, 2*], Zabihi M[2, 3], Aghayan HR[4], Tahamtani Y[2, 5]

1. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
4. Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Diabetes, Obesity and Metabolism, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: y.tahamtani@royan-rc.ac.ir

Objective: During the earliest stage of pancreas organogenesis the pancreatic epithelium evaginates into the surrounding mesenchyme which secrete different growth factors for pancreatic progenitor's (PP) expansion and subsequent differentiation. Mesenchymal cells (MCs) with diverse origins differ in gene and protein expression as well as signaling pathway factors. They also show dissimilar effects in maintenance and differentiation capacity of their organ-matched cells. Co-culture with native pancreatic mesenchyme permits proliferation, self-renewal or differentiation of their epithelial progenitors, demonstrating the importance of cell-cell communications and their interactions during pancreatic organogenesis. Therefore, in this study the impact of human fetal pancreatic mesenchymal cells (hFP-MCs) on further differentiation of human embryonic stem cell derived pancreatic progenitors (hESC-PPs) was examined through a three-dimensional co-culture system.
Materials and Methods: The hESC-PPs differentiation was accomplished from Royan hESC lines. HFP-MCs were isolated from 12-16 week human embryos, cultured and characterized for VIMENTIN and DESMIN mesenchymal markers. Afterwards the cell mixture suspension containing hESC-PPs and hFP-MCs at 1:1 cell ratio were co-cultivated in pancreatic endoderm media in Matrigel for 14 days. The hESC-PPs existence and their ability to differentiate to the endocrine lineage were examined by evaluation of specific markers by immunestaining and quantitative Real-Time PCR analysis.
Results: hFP-MCs showed morphological features and also

VIMENTIN expression similar to bone marrow-derived mesenchymal stem cells (BM-MSCs). However, these cells controversially showed higher expression of DESMIN marker. After co-culture of hESC-PPs with hFP-MCs, generated PP organoids indicated higher expression of NGN3 and INSULIN displaying the acceleration of pancreatic mesenchyme towards beta cell maturation.
Conclusion: Using native mesenchyme in co-culture provides an opportunity for studies on endocrine-niche interactions and developmental processes by mimicking the pancreatic tissue.
Keywords: Pancreatic Differentiation, Pancreatic Fetal Mesenchyme, Embryonic Stem Cells, Pancreatic Progenitor, Organoids

Ps-14: The Influence of Chemical Stimuli of Estrogen on Neurofilament Gene Expression in Endometrial Stem Cells

Hasanzadeh E[1, 2*], Ai J[2], Ebrahimi-Barough S[2], Mahmoodi N[3], Nekounam H[4], Asadpour Sh[5]

1. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Medical Nanotechnology, School of Advanced Technology in Medicine, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran
Email: Hasanzadeh.elham88@gmail.com

Objective: For treatment of neurological injuries, targeted stem cells differentiation into neural phenotypes could be a potential therapeutic approach. Endometrial stem cells (EnSCs) represent a promising cell source for neural tissue engineering with their ability to differentiate into various neural cell types. Estrogen is a hormone that has robust effects on central nervous system (CNS) signal transduction pathways. This hormone provides the induction of growth factors, which are important for neuronal differentiation, and prominent neuro-protection in CNS. The aim of this study is the investigation of estrogen effect on neurofilament (NF-H) gene expression in neural-like cells differentiated from EnSCs.
Materials and Methods: Human EnSCs were isolated and characterized in passage 3 using flow cytometry, adipogenic, and osteogenic differentiation. In order to neural differentiation, cells were exposed to estrogen, epidermal growth factor, fibroblast growth factor-2, retinoic acid, B27 and ITS supplements for 14 days. After neural differentiation, the expression level of NF-H, as a neural-specific marker, was quantified using immunofluorescence and quantitative Real-Time PCR (qRT-PCR).
Results: After 14 days of neural induction, qRT-PCR results showed up-regulation of this neural-specific marker in the mRNA level. Also, immunofluorescence images showed the high expression of NF-H at the level of protein, compared to undifferentiated human EnSCs as the control group.
Conclusion: Estrogen is an important female sex hormone that can affect neuronal differentiation. According to our findings, hEnSCs can be a potential and approachable cell source for enhancing neural regeneration and tissue engineering. Furthermore, our data confirmed the influence of estrogen on the expression of NF-H, as a neural marker, in hEnSCs.
Keywords: Human Endometrial Stem Cells, Neural-Like Cells , Differentiation, Neurofilament (NF-H) ,Estrogen

Ps-15: BMP4 Cannot Be Substituted by Small-Molecule During Germ Cell Differentiation from Embryonic Stem Cells

Hayaei Tehrani RS[1, 2*], Sayahpour FA[1, 2], Esfandiari F[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
Email: fereshtehesfandiari@royaninstitute.org

Objective: Germ cell production from stem cells allows investigation of the involved mechanisms in gametes development with the goal of producing healthy gametes for infertile couples. In this regard, improvement of the protocols for *in vitro* reconstitution of germ cell development from stem cells is in the spotlight of research. Recently, SB4 small molecule has been introduced as a potent agonist for BMP4. These evidences suggest SB4 as a potent candidate to substitute BMP4 (as key inducer of germ cell) during *in vitro* germ cell differentiation for providing a cost-effective protocol.
Materials and Methods: To examine the SB4 as a substitute for BMP4 during *in vitro* germ cell differentiation, after finding non-toxically SB4 concentration for germ cell culture by PI staining (200 μM/ml), germ cells were induced via Hayashi's protocol in the presence of BMP4 or SB4 or both of them. Aggregates were collected for gene expression analysis after 4 days of induction.
Results: Our data showed that the level of Blimp1 expression in SB4-induced aggregates is significantly less, compared to BMP4-induced aggregates ($P<0.05$). Prdm14 expression increased significantly in both SB4- and BMP4- induced cells compared ($P<0.01$), but no significant differences were shown in SB4- and BMP4- induced groups. Also, at the simultaneous presence of BMP4 and SB4, there was significantly increasing levels of Prdm14 but no significant difference in Blimp1 expression compared to BMP4 alone.
Conclusion: It seems that the SB4 could not induce germ cell lineage. However Simultaneous presence of BMP4 and SB4 may improve the establishment of germ cell fate *in vitro* via increasing Prdm14 expression.
Keywords: Infertility, Primordial Germ Cells, BMP4, Small Molecule, SB4

Ps-16: Assessing The Effect of Human Amnion-Derived Mesenchymal Exosomes in Angiogenesis Induction

Hayati D[1*], Valizadeh M[1], Niknejad H[2], Rajabi-Maham H[1]

1. Department of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
2. Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: donya.hayati@gmail.com

Objective: Angiogenesis is the creation of new blood vessels from preexisting ones. How blood vessels interact with cells in their nearby microenvironment is yet to be studied but it might be through exosomes. Recently exosomes have gained a lot of attention to be as a carrier for a variety of macromolecules which control intracellular communication. Human amnion mesenchymal tissues (hAm) have various potentials for therapeutic effects, including angiogenesis. The aim of this study was to evaluate the influence of hAm- mesenchymal-derived exosomes (hAm-Exo) on angiogenesis.
Materials and Methods: hAM was isolated from the placenta by enzymatic digestion and cultured into a serum-free 12 well plate. The condition media were collected after 24 hours and their exosomes were isolated using Exocib kit. The exosomes were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS) and a BCA kit. The angiogenesis activity of hAm-Exo was assessed by the Dorsal Skinfold Chamber in an *in vivo* angiogenesis model.
Results: TEM and DLS showed that exosomes have a size range of about 30 nm with an appearance of cup-shaped. Vessel density, total vessel network length, total branching points and total segments were increased ($P<0.04$) by the compositions of hAm-exo. The size of the wound decreased ($P<0.01$) compared to the control group.
Conclusion: This study demonstrated that condition media from human amnion membrane contained exosomes that can increase angiogenesis when tested in an *in vivo* angiogenic model. With more studies on this matter hAm-Exo could be a great candidate for the treatment of ischemic diseases.
Keywords: Vasculogenesis, Amniotic Membrane, Extracellular Vesicles

Ps-17: Neuroprotective, Neurodifferentiative, and Anti-Neuroinflammatiory Effects of Ferulic Acid

Homayouni Moghadam F[1*], Khajeh-Mobarakeh M[2], Dehghani-Varnamkhasti R[2], Mesbah-Ardakani M[3], Nasr-Esfahani MH[4]

1. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Biology, ACECR Institute of Higher Education, Isfahan, Iran
3. Imam Hossein Hospital of Sepidan, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: f_homayounim@yahoo.com

Objective: Ferulic acid (FA) as a phenolic phytochemical has many medicinal properties that have been proven to have antioxidant and protective effects on various cell types, including neurons. However, studies on its effects on other biological processes, such as inducing neuronal differentiation and the stimulatory effect on the change of microglia phenotype from M1 to M2, are very limited.
Materials and Methods: In a series of studies on PC12 cells, neuronal precursors, and microglia cells, we found that these neurons reacted differently to different concentrations of the compound. In these experiments, we treated PC12, NSC and microglia cells with different concentrations of FA and evaluated the expression of genes and proteins involved in differentiation, survival and inflammation in different laboratory conditions. Western blotting, quantitative real-time PCR, immuno-cytochemical staining, survival measurement with MTT assay and morphological studies were used to confirm the findings.
Results: FA specifically promotes the survival of nerve cells and increases their resistance to oxidative stress by stimulating the expression of Sirtuins, especially SIRT 1 and 7. It also induces differentiation in NSCs toward neurons through the ERK 1/2 signaling pathway. Regarding induction of differentiation, our studies showed that at high concentrations FA shows this property, while at low concentrations it promotes neuroprotection. Regarding the stimulation of microglia, our study showed that FA in low concentrations has the property of inducing the M1 to M2 shift of microglia and thereby reducing the aggressive properties of microglia. In this way, FA acts through suppression of M1-inflammatory genes (IL 1β, IL6, TNFα, OPG) and increasing the expression of M2-anti-inflammatory markers (IL10, IL4, TGFβ).
Conclusion: Our studies show that FA is not just an antioxidant and can have significant effects on inducing differentiation and reducing inflammation in the nervous system.
Keywords: Ferulic acid, Neuron, Microglia, Differentiation, Protection

Ps-18: Generation of Three-Dimensional Renal Micro-Tissue with Co-Culture of Human Pluripotent Stem Cell-Derived Renal Precursors and Supportive Cells

Khoshdel-Rad N[1, 2*], Moghadasali R[1, 2], Aghdami N[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
Email: niloofarkhoshdelrad2@gmail.com

Objective: Directed differentiation of human pluripotent stem cell (hPSC) using a growing number of small molecules and growth factors is required for *in vitro* generation of renal lineage cells. By combining two-dimensional (2D) protocol with a three-dimensional (3D) co-culture system, we established a low-cost and rapid method for generation of a 3D renal model from hPSCs.
Materials and Methods: To determine whether a cost-effective small molecule, inducer of definitive endoderm 1 (IDE1), can be used to replace activin A, we examined gene expression of multiple renal lineage markers in cells differentiated with IDE1. In the following, we employed a 3D co-culture system in which hPSC-derived kidney precursors were co-cultured with endothelial cells and mesenchymal stem cells (named RMEM). hPSC-derived kidney precursors were cultured either alone (renal micro-tissue (RM)) or in co-culture with Human umbilical vein endothelial cells (HUVECs) and bone marrow-derived mesenchymal stem cells (BM-MSCs) for 11 days.
Results: Data showed the expression of nephrogenic markers was significantly increased in cells differentiated with IDE1 compared with cells differentiated using activin A. These data indicated that renal lineage cells can indeed be generated using this alternative approach. Immunofluorescent staining showed the expression of kidney specific markers, as well as endothelial cells that distributed throughout RMEMs. Quantitative Re-

al-Time PCR analysis confirmed a significant increase in gene expression of some renal-specific markers in RMEMs.
Conclusion: These findings demonstrated that renal precursors in co-culture with endothelial cells and mesenchymal stem cells showed greater maturity compared with micro-tissue without these cells.
Keywords: Renal Micro-Tissue, Endothelial Cell, Human Pluripotent Stem Cells, Differentiation, Mesenchymal Stem Cell

Ps-19: Long Term Exposure to Nitric Oxide Caused Cell Cycle Arrest Due to Down-Regulation of Cdk2 and CDK4

Maleklou M[1*], Abnosi MH[2]

1. Department of Biology, Arak University, Arak, Iran
2. Department of Biology (Cell Culture), Arak University, Arak, Iran
Email: mahsamaleklou1@gmail.com

Objective: Nitric oxide (NO) is a free radical and a signaling molecule which controls many cellular and physiological mechanisms such as; blood vessel contraction, blood pressure and immunological response. In previous studies it was shown that the short time treatment of mesenchymal stem cells (MSCs) with sodium nitroprusside (SNP) as an NO releasing agent at low concentration did not affect the cell viability, but caused metabolic imbalance. The aim of this study was to investigate the effect of low concentration of SNP for long time on cell viability, proliferation, cell cycle and expression of the genes involved in cell cycle.
Materials and Methods: MSCs after the third passage was treated every 1 hour in each 72 hours with 100 µM of SNP. After 5, 10, 15 and 20 days, the viability and proliferation of the cells was estimated using MTT, Tripan blue and PDN, CFA method. In addition, we used flow cytometry to study the cell cycle whereas the profile of the proteins was evaluated using electrophoresis. The expression of Raf1, Cdk2, Cdk4, P53 and GAPDH genes involves in the cell cycle was determined using quantitative real-time PCR. Also, we checked the effect of the repeated passages on the control and treated of MSCs growth.
Results: Cell treated with SNP caused the reduction of viability at 5, 10, 15 and 20 days. The same treatment caused the number of the colonies to reduce significantly at all treatment times, but no changes were observed in diameter of the colonies. In addition, the cell cycle was 93% arrested at G1 stage following SNP treatment at day 20. In the MSCs treated with SNP, the expression of the Cdk2 and Cdk4 was reduced; whereas the expression of P53 was elevated and the Raf1 expression remained the same. In the treated cells we observed changes in the density of some polypeptides, where two polypeptides with the molecular weight of 16.73 and 13.40 were expressed only in the treated group, the cell treated with SNP tolerated up to 11 passages and the changes included morphological abnormalities, enlargement continuously from the 10 passage in both groups with increasing passage. Finally, it showed a decrease in growth and ultimately proliferation arrest.
Conclusion: This study showed that the long time treatment with NO at low concentration may cause the reduction of MSCs resources on one side and the cell cycle arrest at G1 stage due to elevation of P53 expression and reduction of Cdk2 and Cdk4 expression on the other side. Also NO period at low concentration in long time might cause the stem cell reservoir to reduce. In addition, this compound in long time causes replicative senescence in bone marrow MSCs.
Keywords: Cell Cycle, Mesenchymal Stem Cells, Cell Viability, Cell Proliferation, Nitric Oxide

Ps-20: Improvement of Cardiac Function Following Menstrual Blood Stem Cells Administration by Prevention of Cartilaginous and Osseous Development in Rat Model of Myocardial Infarction

Manshori M[1*], Golshahi H[2], Darzi M[2], Naderi N[3], Shirazi A[4], Arabian M[3], Montazeri S[2], Eghtedar Doost M[2], Kazemnejad S[2]

1. Faculty of Veterinary Medicine, Shahrekord University, sharekord, Iran
2. Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
3. Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
4. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: s.kazemnejad@ari.ir

Objective: Menstrual blood stem cells (MenSCs) are introduced as cells with high therapeutic potential in regenerative medicine. To prove the efficiency of MenSCs administration in myocardial infarction (MI) we assessed the effects of intramyocardial injection of MenSCs accompanied by their derived conditioned medium (CM) on cardiac function in MI rat model.
Materials and Methods: All animals received human care according to the criteria outlined in declaration of Helsinki. The study was approved by bioethics committee of Avicenna Research Institute. 40 rats were randomly divided into four groups including sham, MI (PBS), CM, and MenSCs + CM. Echocardiography and histopathological evaluation were performed at day 7 and 28 days post-MI.
Results: The results demonstrated significant improvement of cardiac function in terms of ejection fraction (EF) and fractional shortening (FS) after MenSCs + CM injection at day 7 ($P<0.05$) and day 28 ($P<0.001$) compared to MI group. In addition, transplantation of MenSCs + CM resulted in higher FS and EF compared to CM-received group ($P<0.05$ and $P<0.05$ respectively) at day 28. Histological and immunohistochemical analyses indicated that administration of MenSCs + CM led to the survival of cardiomyocytes, modulate inflammatory phase, promote neo-angiogenesis, prevent the formation of metaplastic changes and mitochondrial transfer from MenSCs to cardiomyocytes and non-cardiomyocytes. Moreover, MenSCs evoked inhibition of nuclear factor-κB (NF-κB) expression, bone morphogenetic protein-2 (BMP-2) up-regulation and amplifying osteocalcin signaling in preserved cardiomyocytes.
Conclusion: MenSCs accompanied by CM ameliorated the function of cardiac, prevented metaplastic development, and spurred cardiomyocyte survival following MI.
Keywords: Myocardial Infarction, Menstrual Blood Stem Cells, Conditioned Medium, Metaplasia, Mitochondrial Transfer

Ps-21: Nanofibrous Piezoelectric PVDF-Graphene Scaffolds for Cardiac Tissue Engineering

Mashayekhan Sh[1*], Golafshan E[2], Simchi A[3],, Shokrgozar MA[4]

1. Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
2. Institute for Nanoscience and Nanotechnology, Sharif University

of Technology, Tehran, Iran
3. Department of Materials Science and Engineering, Sharif University of Technology, Tehran, Iran
4. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
Email: simchi@sharif.edu

Objective: Piezoelectric polymer-based nanocomposites with fibrous structure are emerging materials for cardiac tissue engineering due to their electroactive properties and the ability to mimic the fibrillary structure of the native cardiac microenvironment.
Materials and Methods: We present a novel hybrid poly(vinylidene fluoride) (PVDF)-graphene nanocomposites with high piezoelectric response, tunable mechanical properties, and improved cell adhesion and proliferation. To attain homogeneous distribution of graphene nanosheets in the polymer matrix, reduced graphene (rGO) nanosheets are functionalized with poly(ethylene glycol) (PEG). Cardiac scaffolds are prepared by electrospinning to attain unwoven fibers with an average diameter of 240 nm and high structural porosity.
Results: The effects of PEGlayted rGO nanosheets (up to 7wt.%) on the electrical and biological properties of the nanofibrous scaffolds are presented. The attachment and spreading of L929 cells on the scaffolds are shown. The improved cell proliferation on the fibrous PVDF nanocomposite containing 1% rGO is also demonstrated.
Conclusion: The potential application of the developed scaffolds for cardiac tissue engineering is presented and discussed.
Keywords: Cardiac Tissue Engineering, Piezoelectricity, Carbon Nanostructure, Electroactive Polymer, Cell Attachment

Ps-22: Reinforcement of Kartogenin Incorporated Extracellular Matrix Derived Hydrogel Using Three-Dimensional Printed Microfibers

Mashayekhan Sh*, Mohsenifard S

Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
Email: mashayekhan@sharif.edu

Objective: The avascular nature of cartilage limits its self-renewal ability. Driving or sport accidents are factors that necessitate the need for local cartilage treatment. Despite the recent numerous attempts performed in the field of cartilage tissue engineering to find a suitable solution for the repair, the challenges are remained and novel clinical methods are needed.
Materials and Methods: This study aimed to reach the mechanical properties of the native articular cartilage by reinforcement of kartogenin (KGN)-incorporated extracellular matrix (ECM)-derived hydrogel using three-dimensional (3D) printed microfibers made of 90% (w/w) polycaprolactone (PCL) and 10% (w/w) starch. The architecture of the printed fibers was inspired by the thickness of collagen fibers in cartilage tissue. The frameworks were 3D printed with varied strand size and strand spacing for optimizing the mechanical properties of the construct. The hydrogel in the form of interpenetrating network (IPN) was made from alginate, cartilage ECM, β-cyclodextrin (β-CD), and KGN. The aim of modifying the alginate with β-CD was to provide a host-guest interaction between KGN and β-CD to have a sustained release behavior of KGN from the hydrogel.
Results: H-NMR spectroscopy revealed that β-CD was grafted successfully to alginate. The results revealed that the controlled release of KGN could be achieved using alginate-graft-β-CD hydrogel. Moreover, the compression strength of the optimized composite hydrogel was comparable to the mechanical strength of the native cartilage.
Conclusion: The ongoing studies are focused on inducing the differentiation of encapsulated mesenchymal stem cells within the hydrogel to chondrocytes.
Keywords: Kartogenin, Three Dimensional-Printing, Cartilage Tissue Engineering

Ps-23: Design and Fabrication of Mineral-Based Porous Microcarrier for Bone Tissue Engineering

Mashayekhan Sh*, Hajiabbas MA

Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
Email: mashayekhan@sharif.edu

Objective: Currently, using bicompatible and injectable polymeric microcarriers as one of the efficient methods to transfer cells and active agents has gained much attention for bone regenerative medicine. However, they have some drawbacks such as weak mechanical stability and lack of mineral materials, which are the major ingredients of the bone tissues. Accordingly, it is expected that mimicking the chemical and physical structure of bone tissues could be valuable in their medical applications.
Materials and Methods: Herein, a new porous biodegradable microcarrier (MCs) made of silk fibroin-oxidized alginate-bioactive glass was fabricated by electrospraying method. Response surface methodology (RSM) was used to study the quantitative influence of process parameters, including blend ratio, voltage, and syringe pump flow rate on MCs diameter, porosity and density and the optimized condition for MCs fabrication was proposed.
Results: Scanning electron microscopy (SEM) analysis revealed that the prepared MCs have a spherical shape with the size of 500 μm showing a highly porous structure. Incorporation of bioactive glass in the MCs ingredients provided suitable bioactivity for osteogenesis application.
Conclusion: Such porous MCs may be considered as promising injectable cell carriers for bone tissue engineering application.
Keywords: Oxidized Alginate, Silk Fibroin, Bioactive Glass, Microcarrier, Bone Tissue Engineering

Ps-24: *In Vitro* Evaluation of Acellular Skeletal Muscle-Derived Hydrogel Containing 5-Azacytidine on The Behavior of Muscle-Derived Stem Cells

Mirza Ahmadi B[1*], Talkhabi M[1], Rajabi S[2]

1. Animal Sciences and Marine Biology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran
2. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Emails: m_talkhabi@sbu.ac.ir, srajabi@royaninstitute.org

Objective: Finding new and appropriate biomaterials/scaffolds is one of the most important aspects in skeletal muscle tissue engineering. Among the biomaterials, decellularized skeletal muscle-derived biomaterials (e.g. hydrogels and scaffolds) are a promising strategy for improving muscle tissue engineering. Moreover, regulatory factors such as 5-azacytidine (a DNA

meythltransferase inhibitor) can induce myogenesis in different type of stem cells, as well as it can regulate viability, migration and secretion. Here, we studied the effect of 5-Aza incorporated muscle-derived hydrogel in muscle regeneration *in vitro*.
Materials and Methods: Wistar rat skeletal muscles were decellularized by sodium dodecyl sulphate (SDS 1%). DNA content and histological analysis was carried out on acellular and native muscle tissues. Then, the muscle extra cellular matrix (ECM) was enzymatically digested using pepsin and then muscle-derived hydrogel was prepared. 5-azacytidine (5 µm) was incorporated into the hydrogel and muscle-derived stem cells (MDSCs) were cultured within the hydrogels. The viability of MDSCs was analyzed using Live/Dead staining.
Results: DNA content analysis, Masson's trichrome (MT), Hematoxylin and eosin (HandE) staining and scanning electron microscope (SEM) confirmed the cell elimination and maintenance of the ECM structure after decellularization. Live/Dead assay showed that hydrogel incorporated with 5-Aza supports MDSCs viability *in vitro*.
Conclusion: Skeletal muscle-derived hydrogel along with 5-azacytidine supports MDSCs viability. In addition, the use of myogenic chemical such as 5-azacytidine and muscle-derived biomaterials might be a promising and useful approach for clinical applications.
Keywords: 5-Azacytidine, Muscle-Derived Stem Cells, Hydrogel, Acellular Skeletal Muscle

Ps-25: Amelioration of Diabetic Wound Using Menstrual Blood Stem Cells Seeded on Bilayer Scaffold Composed of Amniotic Membrane and Silk Fibroin in Mouse Model

Mirzadegan E*, Saffarian Z, Golshahi H, Darzi Maryam, Khorasani S, Kazemnejad S

Nanobiotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: S.kazemnejad@avicenna.ac.ir

Objective: Recently, we fabricated a bilayer scaffold composed of amniotic membrane and silk fibroin in aim for skin regeneration which was characterized physically, biochemically and biologically *in vitro* and *in vivo*. In this study, we evaluated the efficiency of menstrual blood stem cells (MenSCs) seeded on the fabricated scaffold in healing of diabetic wound in animal model.
Materials and Methods: The 75 male diabetic C57/BL6 mice were equally divided into five groups including amniotic membrane group, no treatment group, scaffold group, MenSCs-seeded scaffold group and group constituted of foreskin- derived keratinocytes/fibroblasts seeded on scaffold. All animals received humane care in according to the criteria outlined in the Declaration of Helsinki. The study was approved by bioethics committee of Avicenna Research Institute. The excisional bilateral full thickness circular wounds were created in the dorsal dermis of diabetic mice. Donut-shaped silicon splints were utilized to prevent wound contraction in mouse skin and simulate re-epithelialization. The healing of diabetic wounds in each group was evaluated at 3, 7 and 14 days after their treatment. The healing progression measurements, quantification of dermal and epidermal hypertrophy and quantification of immunofluorescence staining assays were processed in ImageJ software.
Results: The gross morphology, epithelization and histologic changes in regenerated tissue in MenSCs-seeded bilayer nanofibrous scaffold group showed the most similarity to intact tissue. Moreover, the wound healing was typically faster in MenSCs-seeded scaffold group compared to other diabetic groups. Immunofluorescence staining of mouse skin depicted higher levels of CD31 and von Willebrand factor (VWF), higher ratio of M2/M1 macrophages, higher expression of involucrin as a keratinocyte marker and higher levels of neural marker in the MenSCs-seeded bilayer nanofibrous scaffold group in comparison with other treatment groups.
Conclusion: The dramatic effects of MenSCs as a promising adult stem cell population accompanied by significant impact of fabricated bilayer scaffold sounds to mediate a promoting and appropriate circumstance on wound healing and skin regeneration.
Keywords: Wound Healing, Diabetic Wound, Chronic Inflammation, Angiogenesis, Menstrual Blood Stem Cells

Ps-26: The Effect of Nanocomposite Scaffold on Spermatogonial Stem Cells Proliferations

Moeinzadeh A[1, 2*], Mosleh HR[1, 2], Bagher Z[2], Koruji M[1, 2], Ashtari B[3], Shabani R[1, 2]

1. Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Department of ENT and Head and Neck Research Center, The Five Senses Institute, Hazrat Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
3. Department of Medical Nanotechnologhy, Faculty of Advanced Technology in Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: Shabani.r@iums.ac.ir

Objective: Some male survivors of childhood cancer are in distress from azoospermia. Spermatogonail stem cells (SSCs) isolation and purification are really important. Testicular organoids and three-dimensional (3D) scaffolds are possible applications in treatment of male infertility. 3D nanofibers scaffolds play important role in cell culture, and due to these scaffolds provide a microenvironment similar to extracellular matrix for proliferation and self-renewal of cells. The present study aimed to evaluate the efficiency of 3D microenvironment containing the chitosan-alginate –Graphene oxide nanocomposites for cell culture improvement and proliferation of spermatogonial cells.
Materials and Methods: Spermatogonial cells were cultured and divided into 3 culture groups: 1. Control (culture in basic media), 2. SSC culture in CA/GO30µg scaffold, and 3. SSC culture on alginate-chitosan. The identity of the cultured cells was confirmed by flowcytometry (ckit and GFRα1).The Scaffolds were analyzed using FTIR, XRD, and scanning electron microscopy (SEM) to observe surface topography and the morphology .Cytotoxicity of scaffolds was assayed at 24 hours, 72 hours and one week after seeding using MTT assay. The stem cells related markers for SSCs (Id4, GFRα1 and PLZF) were detected on all experimental groups by quantitative Real-Time PCR (qRT-PCR).
Results: These results showed that SSCs can easily attach and proliferate on the substrates. The CA/GO30µg scaffold was biocompatible, as evidenced by the MTT assay. SSC that were seeded onto the surface of the scaffold exhibited good proliferation. The quantity of proliferations marker significantly increased compared with control group. The qRT-PCR results confirmed that GO-based chitosan-alginate scaffold may provide an ideal environment for SSC proliferations.
Conclusion: We conclude that SSCs culture in the group CA/

GO30µg has a potential use for SSCs proliferation *in vitro*. This 3D scaffold is applicable for culturing and encapsulation of SSCs.

Keywords: Spermatogonial Stem Cells (Sscs), Alginate Chitosangraphene Oxide Nanocomposites, Alginate, Chitosan, Cell Culture

Ps-27: Anti Neuro-Inflammatory Effects of Ferulic Acid by Induction of M2 Shift in Microglia

Moghimi Khorasgani A[1, 2*], Homayouni Moghadam F[2], Nasr-Esfahani MH[2]

1. Department of Biology, ACECR Institute of Higher Education, Isfahan, Iran

2. Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Email: f_homayounim@yahoo.com

Objective: Ferulic acid (FA) is a natural phenolic antioxidant, which can exert also several other beneficial effects to combat neuro-inflammation and neuro-degeneration in Alzheimer's and Parkinson diseases. Microglial cells are innate immune cells that reside within the central nervous system (CNS). Activated M1 microglia mediates some aspects of neuro-degeneration in Alzheimer's disease, while M2 microglia which is known as good microglia reverses these toxic effects of M1 microglia (bad microglia). In this study we examined the effects of FA, with strong anti-oxidative and anti-inflammatory activities on microglia cells.

Materials and Methods: In this experimental study, microglia were isolated from 2-day-old male mouse pup's brain and these cells were treated with various concentration of FA (50, 100, 250, and 500 µg/ml) for 24 hours. We assisted the following cell treatments anti-oxidant and Anti-inflammatory genes expression.

Results: The results revealed that FA (50µg/ml) significantly increased expression of anti-inflammatory and antioxidant genes expression. It also had effects on cell morphology and cell viability.

Conclusion: Based on our previous reports FA exerted neuroprotective and neuro-differentiative properties that make it a suitable choice for treatment of Parkinson and Alzheimer's diseases. Our results indicate that it also has positive effects on microglial cells to activate them to express anti-inflammatory and antioxidant markers.

Keywords: Microglia Cell, Ferulic Acid, Anti-Inflammatory, Antioxidant

Ps-28: A Niosomal Thymol Loaded Collagen/B-TCP Scaffold for Addressing Bone Infection Control

Najafloo R[1*], Baheiraei N[2], Imani R[3]

1. Department of Bioinformatics, Faculty of Interdisciplinary Science and Technology, Tarbiat Modares University, Tehran, Iran

2. Tissue Engineering and Applied Cell Sciences Division, Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

3. Department of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran

Email: n.baheiraei@modares.ac.ir

Objective: Despite the continuous progression in surgical techniques and antibiotics therapies, the simultaneously bone repair and inhibit infection is difficult. Among antimicrobial agents, thymol (2-isopropyl-5-methyl phenol) has revealed favorable antimicrobial activity in many reports. This study aimed to develop a novel niosomal nanocarrier embedded into the collagen/β-TCP scaffold for local delivery of thymol as a natural antibacterial reagent.

Materials and Methods: Niosomal nanocarrier loaded with thymol was synthesized by lipid film hydration technique. Morphology and size of the synthesized niosomes were evaluated by Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS), respectively. Collagen/β-TCP scaffold incorporating niosomal thymol was also prepared using the freeze-drying technique. Thymol release from scaffolds was calculated with the Uv-visible method. The viability and morphology of L929 fibroblast cells were investigated on niosomal containing scaffolds, and the antimicrobial activities of the prepared samples were assessed by agar diffusion method against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus.

Results: The synthesized niosome showed uniformed size distribution and high efficacy of loading with being successfully incorporated into the composite scaffolds. The release profile of niosomal thymol from the composite scaffolds exhibited a sustained manner. Niosome added scaffolds showed significantly higher values of cell viability, and excellent cell adhesion and spreading when compared to that for other groups. Also, niosomal scaffolds showed greater effectiveness of antibacterial activity towards all three types of bacteria with higher activity against Staphylococcus aureus.

Conclusion: The results of this study highlight the potential of niosomal-thymol loaded Col/β-TCP scaffold as an antibacterial bone substitute for possible osteomyelitis treatment.

Keywords: Bone Infection, Scaffolds, Antibacterial, Thymol, Tissue Engineering

Ps-29: Bone Marrow-Derived Clonal Mesenchymal Stromal Cells with Smaller Size Have Higher Proliferation Capacity

Pakzad M[1,2*], Abbasi F[1], Hajizadeh-Saffar E[1], Hassani SN[1], Baharvand H[1, 2]

1. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

2. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran

Email: pakzad_m@ymail.com

Objective: Production of clonal mesenchymal stromal cells (cMSCs) from a single colony-forming unit is among the best prosperous methods to obtain a homogenous population of MSCs with the endeavor of high passage number. To find the best passageable clones we have to passage tens of clones for more than two months, which is so time and cost consuming. Therefore, we put emphasis to find a factor that can predict the high-proliferative clones.

Materials and Methods: In this study, 93 clones were isolated from the bone marrow aspirations of three healthy donors with signed consent. After screening, the best-selected clones were assessed by immunophenotype, differentiation potential, karyotype, and tumorgenicity. We profiled the cell size of different clones at passage number two using an automated cell counter

(TC20). The histograms drawn by this machine were carefully analyzed.
Results: Based on our data, clones with the cell size in the range of 10-14, 14-18, 18-22, 22-26, and 26-30 micrometer could reach to passage number 12-15, 9-12, 6-9, 3-6 and 2-4, respectively. The data shows that there is a significant reverse relationship between the size of cMSCs in passage number two and the passage ability of the clones. Here, four clones could reach passage number 15 which all of them were at the range of 10-14 micrometers. Morphology, expression of standard MSC markers, and differentiation potential of these cMSCs at passage number 15 were normal. Moreover, their karyotype has shown no abnormality and no tumors were made in three months after injection to nude mice.
Conclusion: Accordingly, cell size can be used as a novel robust criterion to predict the best proliferative cMSCs in the cell manufacturing units.
Keywords: Clonal Mesenchymal Stromal Cells, Proliferation Capacity, Cell Size

Ps-30: Pericardium Biological Membrane for Periodontitis

Pourmostafa A[1*], Samanipour R[2], Tavakoli AH[1], Marzban A[1]

1. Department of Research and Development, Iranian Tissue Product Co, Tehran, Iran
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Samanipour.med@gmail.com

Objective: Heart pericardium membrane allografts are used as a periodontal resorbable membrane which is placed in the surgical site to prevent epithelium from growing into the healing site before bone formation and growth of periodontal ligament. Pericardium tissue is shown to be a collagen abundant extracellular matrix after decellularization.
Materials and Methods: In order to obtain an intact pericardium collagen membrane, it should be processed with a technological principle. To avoid any probable disease transmission from a donor, the human pericardium is supplied, procured and sterilized under the guidelines of the American Association of Tissue Banks (AATB). In this research, we processed the human pericardium with a mild chemical and enzymatic treatment, and after sterilization and lyophilization, the structure morphology remained, is observed by the Scanning electron microscope.
Results: It demonstrates a remarkable parallel fibrous structure and the surface that is suitable for cell reseeding and immigration. Furthermore, histochemical staining by Masson's Trichrome (MT) used to evaluate collagen presence and intensity. To investigate the decellularization efficiency, DNA quantification established to measure the amount of viable cells in pericardium membrane allograft. By comparing the native tissue and decellularized membrane, a 91% decrease of DNA content was resulted and showed that the majority of DNA is removed from the tissue and only small amounts are remained.
Conclusion: The removal of almost the entire cells which keeps native collagen structural will reduce the possibility of immune responses for applying the decellularized pericardium for the regeneration of all the components of the periodontium, mainly based on guided tissue regeneration (GTR) and guided bone regeneration (GBR).
Keywords: Decellularization, Pericardium, Collagen

Ps-31: A Method for *In Vitro* Differentiation of Rabbit Adipose Derived-Mesenchymal Stromal/Stem Cells into Cardiomyocyte-Like Cells

Sadeghian Chaleshtori S[1, 2*], Mokhber Dezfouli MR[1, 2], Shaban Malekshah D[3], Komam A[3], Jabbari Fakhr M[2, 4]

1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2. Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Tissue Engineering, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran
4. Institute of Biomedical Research, University of Tehran, Tehran, Iran
Email: s.sadeghian@ut.ac.ir

Objective: Cardiac diseases such as acute myocardial infarction and ischemic cascade can rapidly develop and finally result in the loss of cardiomyocytes and congestive heart failure and are a reason for many deaths every year worldwide. Mesenchymal stromal/stem cells (MSCs) can differentiate into mesenchymal lineages, including cardiac cell types. This capacity is a promising therapeutic strategy for cell-based therapies to replace current invasive techniques.
Materials and Methods: Different chemical substances, growth factors, and cytokines regulate the cardiomyocytes differentiation. We investigated the effect of new combination of differentiation materials on the third-passage of MSCs derived from adipose tissue. Firstly, these cells were pre-treated with 5-Azacytidine which is an important chemical inducer for demethylation of DNA, in concentration of 5 and 10 µM for two days respectively. Then cells were cultured with 10 ng/ml basic fibroblast growth factor (bFGF) and 50 µg/ml ascorbic acid for 21 days to induce cardiomyocytes differentiation of MSCs. For characterization, quantitative real-time PCR (qRT-PCR) and Flow cytometery were performed.
Results: Morphologically, cardiomyocytes are observed as rod-shaped cells that attach to adjacent cardiomyocytes via phase contrast light microscopy. Flow cytometery analysis displayed that increased expression cardiac troponin T and connexin 43/GJA1 and quantitative real-time PCR analysis showed increased expression of cardiac markers such as GATA4, NKX2.5, desmin and cardiac intracellular gap junction protein (connexin 40 and 43) when compared with negative control.
Conclusion: Our results demonstrated that this differentiation protocol (5-azacytidine combined with ascorbic acid and bFGF) improve cardiomyocyte-like cells differentiation from MSCs. Stem cell differentiation-based therapies have emerged as a promising therapeutic tool for improving cardiac regeneration and function.
Keywords: Differentiation, Adipose Derived-Mesenchymal Stromal/Stem Cells, Cardiomyocyte, Rabbit,

Ps-32: Evaluating The Anti-Inflammatory Effect of Conditioned Medium Derived from Endometrial Stem Cells in Endometriosis Patients

Sahraei SS[1*], Kowsari A[2], Seyed Ebrahimi SR[3], Khodavandpour Z[2], Davoodi Asl F[2], Sheikholeslami A[2]

1. Department of Reproductive Biology, The Academic Centre for Education, Culture and Research, Qom, Iran
2. Department of Mesenchymal Stem Cells, The Academic Centre for Education, Culture and Research, Qom, Iran

3. Department of Anatomical sciences, Isfahan University of Medical Sciences, Isfahan, Iran
Email: azareslami10@gmail.com

Objective: Although, the endometriosis is a benign inflammatory disorder, it mainly causes complications. Thus, trials for better diagnosis and treatment seem necessary. It is supposed that mesenchymal stem cells derived from menstrual blood (MenSCs) in endometriosis patients are different from that of healthy women and so, they are responsible for the creation of the disease. According to the high expression of inflammatory factors in MenSCs of endometriosis patients, it was supposed that the conditioned medium of these cells from healthy women can affect the expression of inflammatory genes. The aim of this study was evaluating the expression of inflammatory genes in endometriosis patients under treatment of conditioned endometrial from MenSCs of healthy women.
Materials and Methods: Menstrual blood samples (1-2 ml) were collected from endometriosis women and healthy women as control group, in the age range of 22-35 years. Isolation of MenSCs from two groups was performed by the Ficoll-Paque density-gradient method and then endometriosis-derived MenSCs were treated with conditioned medium from control group in passage three. The expression of inflammatory genes including IL-1α, COX-2, TNF-α, NF-kB and HIF-1α were assessed using quantitative real-time PCR.
Results: Endometriosis-derived MenSCs were morphologically different from healthy MenSCs and also showed higher expression of CD10 marker, which experienced significant decrease in CD10 marker after 4 days' treatment with conditioned medium of healthy MenSCs. Also, after treatment, Endometriosis-derived MenSCs showed significant decrease in TNF-α and NF-kB expression but no significant decrease in HIF-1α.
Conclusion: Down-regulation of inflammatory genes in endometriosis-derived MenSCs under treatment of conditioned medium derived from healthy MenSCs can be considered as an effective approach for the treatment of endometriosis which needs more investigations.
Keywords: Menstrual Blood-Derived Stem Cells, Endometriosis, Inflammatory Genes

Ps-33: Evaluation of The Effect of Metformin on The Proliferation Rate and Antioxidant Enzymes Activity of Mesenchymal Stem Cells

Sistani S*, Ghorbanian MT

Department of Cellular and Molecular Biology, School of Biology and Institute of Biological Sciences, Damghan University, Damghan, Iran
Email: s.sistani25@gmail.com

Objective: Stem cells are used in tissue engineering due to self-renewal properties and ability to differentiate into different lineages. Much attention has been paid to improving culture conditions, increasing the capacity and capability of mesenchymal stem cells (MSCs) for cell transplantation. In this study, the viability rate and antioxidant enzyme activity at passage 3 of metformin-treated rat bone marrow mesenchymal stem cells (rBM-MSCs) were evaluated.
Materials and Methods: rBM-MSCs were collected form tibia and femora of rat and then the extracted stem cells were cultured in 96-well plate, and treated with 1, 5, 10, 15 and 50 µM of metformin for 24 hours. Then the cell proliferation was measured by MTT assay. SOD enzyme activity (prevention of photochemical reduction of NBT), GPX (NADPH consumption) and MDA levels were measured in the cells.
Results: The results of this study indicate that the proliferation rate at 15 and 50 µM metformin showed a significant increase compared to the other treated and control groups. Metformin-treated cell groups showed higher antioxidant capacity than the control group. The markers of lipid peroxidation were decreased in the treated group with metformin.
Conclusion: Therefore, metformin may be suggested as a pretherapist to strengthen MSCs before transplantation. In addition to using metformin in other fields, it can also be used to reduce oxidative stress.
Keywords: Antioxidant Enzymes, Metformin, Mesenchymal Stem Cells

Ps-34: The Effect of Betaine on Osteogenic Differentiation of Human Adipose-Derived Mesenchymal Stem Cells *In Vitro*

Tabatabai TS[1*], Haji Ghasem Kashani M[1], Maskani R[2], Nassiri M[1]

1. Department of Cellular and Molecular Biology, School of Biology and Institute of Biological Scienes, Damghan University, Damghan, Iran
2. Department of Medical Sciences, Faculty of Paramedical Sciences, Shahroud University, Shahroud, Iran
Email: t.sadat 69@gmail.com

Objective: Human adipose- derived stem cells (hASCs) are affected by many biochemical and biophysical stimuli *in vivo*. Betaine (a dietary supplement) is a biochemical stimulant that promotes the osteogenic differentiation by increasing the entry of calcium into the cell. In this study, the effect of Betaine on the expression of osteogenic genes was evaluated.
Material and Methods: hASCs were extracted from abdominal adipose tissue, and stem cells were confirmed by flow cytometry. The experimental groups were: cultured cells in osteogenesis differentiation medium (positive control), in osteogenesis differentiation medium and Betaine 10 mM (BET + OD), in Betaine (BET) and negative control. On day 21, alkaline phosphatase (ALP) activity and expression of osteogenic genes (RUNX2 and OCN) were assessed by Real time PCR.
Results: A significant increase in ALP activity in BET group was detected compared to OD + BET and positive control. On day 7, there was a significant increase in ALP activity compared to 14-day treatment. There was a significant decrease in the mRNA level of RUNX2 in BET group compared to positive control. The expression of OCN gene in BET group was significantly increased compared to the OD + BET, while a significant increase of this gene was seen in positive control compared to OD + BET. There was no significant difference between BET and positive control.
Conclusion: The induction of hADSCs with Betaine increased ALP activity and OCN gene expression and decreased RUNX2 gene expression. The effect of Betaine on OCN gene expression was similar to osteogenesis differentiation medium. Betaine had effective role on ALP activity at an earlier time post induction.
Keyword: Human Adipose-Derived Stem Cells, Betaine, Osteogenic Genes, Real Time PCR,

Ps-35: Preclinical Toxicity Study of Clinical Grade Allogeneic Human Bone Marrow-Derived Clonal Mesenchymal Stromal Cells

Tayebi B[1, 2*], Babaahmadi M[1, 2], Pakzad M[3], Hajinasrollah M[3], Kamali A[4], Hassani SN[2, 3], Baharvand H[2, 3], Hajizadeh-Saffar E[1, 2, 5]

1. Department of Applied Cell Sciences, Faculty of Basic Sciences and Advanced Medical Technologies, Royan Institute, ACECR, Tehran, Iran
2. Advanced Therapy Medicinal Product Technology Development Center (ATMP-TDC), Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. AO, Research Institute Davos, Davos, Switzerland
5. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: Hajizadeh.ehs@royaninstitute.org

Objective: Mesenchymal stromal cells (MSCs) have opened a new window for the treatment of inflammatory and non-inflammatory diseases. Nonetheless, its clinical applications require rigorous control and monitoring procedures to ensure compliance with all Good Manufacturing Practices (GMP) principles. For bench-to-bedside development of these newly-emerging therapeutic products, they should pass different stages from *in vitro* characterization to pre-clinical studies, in addition to clinical trial phases to ensure product safety and efficacy. In this regard, a robust pre-clinical study is critical to ensure product safety. The aim of this study was to determine the toxicity effects of local and systemic injection of human bone marrow-derived clonal mesenchymal stromal cells (hBM-cMSCs) in acute and sub-chronic periods.
Materials and Methods: In the present study, hBM-cMSCs were characterized by defined criteria of ISCT for MSCs. The safety and toxicity of hBM-cMSCs population, produced in GMP compatible conditions, were assessed in both sexes of Sprague Dawley (SD) rats through systemic interavenous route and local injection in intervertebral disc (IVD). Changes in body weight, food and water consumption which are important variables in product toxicity tests were assessed. After passing the expected time periods (14 days for the acute period and 90 days for the subchronic period) and sacrificing the rats, laboratory tests and histopathology of target tissues were performed on designated specimens. Also tumorigenicity was checked in nude mice.
Results: Single injection of hBM-cMSCs through intravenous or IVD route during the 14 and 90-days period did not cause significant changes in clinical symptoms and laboratory data of all animals. *Ex vivo*-expanded and cryopreserved hBMCMSCs did not induce tumor formation in nude mice.
Conclusion: The results suggest that local and systemic administration of allogeneic or xenogeneic hBM-cMSCs in both sexes of SD rats does not impose tumorigenicity and/ or toxicity in acute and subchronic periods.
Keywords: Toxicity, Bone Marrow Clonal Mesenchymal Stromal Cells, Good Manufacturing Practices, Tumorigenicity

Ps-36: The Small Molecule Enoxacin Suppresses The Growth and Invasiveness of Esophageal Cancer Cells

Torabi P[1, 2*], Miri R[3], Moradi Sh[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, , Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
3. Cancer Research Center, Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran
Email: sharif.moradi@royaninstitute.org

Objective: Esophageal cancer (EC) is one of the deadliest cancers worldwide. The global down-regulation of microRNAs, i.e. non-coding RNAs involved in post-transcriptional gene regulation, is observed in many cancer types including EC. We therefore hypothesized that the microRNA-enhancing compound enoxacin might impair the growth of EC cells.
Materials and Methods: EC cells were cultured in RPMI medium containing 10% FBS. The cells were analyzed three days after treatment with enoxacin (124 μM). To minimize the unwanted increase in pro-tumor microRNAs by enoxacin, one major pro-EC miRNA, i.e. miR-106a, was inhibited using antagomiRs. Viability assays were performed using MTS and Live/Dead staining kits. Cell cycle analysis was done using flow cytometry. The anti-miR-106a was delivered using DharmaFECT1. Human EC tumor tissues were obtained from Imam Khomeini Hospital and exposed to enoxacin for 10 days.
Results: We found that enoxacin inhibited the viability and cell cycling of EC cells (KYSE-30 and YM-1 cell lines). It also suppressed the migration and colony-formation ability of EC cells. Notably, blocking miR-106a activity significantly reduced the viability, cell cycling, migration and colony formation of EC cells. Moreover, the combination of enoxacin and anti-miR-106a induced a decrease in the growth of EC cells that was much more prominent than either of them alone. Finally, we treated tumor samples from EC patients with enoxacin *ex vivo* and found that the tumor tissues were negatively affected by exposure to enoxacin (with or without anti-miR-106a).
Conclusion: Enoxacin, alone or in combination with miR-106a inhibitor, inhibits the growth and migration of EC cells both *in vitro* and *ex vivo*.
Keywords: microRNA, Cancer Cells, Enoxacin, Tumorigenesis, miR-106a

Ps-37: Using Microwell Chip for Scalable Generation of Pancreatic Aggregates

Zabihi M[1, 2*], Ghezelayagh Z[2, 3], Zarkesh I[4], Vosough M[5], Tahamtani Y[2, 6]

1. Department of Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center , Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Developmental Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
4. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
6. Department of Diabetes, Obesity and Metabolism, Cell Science

Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: yaserta@yahoo.com

Objective: For the production and bio-banking of organoids in large quantities for drug screening and cellular therapies, well-defined procedures for scalable production are required. As well, based on previous studies, co-culture with native pancreatic mesenchyme promotes self-renewal and expansion of their epithelial progenitors. Therefore, in this study we used a microwell platform as a high throughput three-dimensional (3D) culture system where human embryonic stem cells derived pancreatic progenitors (hESC-PPCs) can be co-cultured with human fetal pancreatic mesenchyme (hFP-MCs) for large-scale generation of pancreatic aggregates. The advantage of this platform compared to conventional suspension culture is its reproducibility.
Materials and Methods: Non-adherent agarose microwell chip was fabricated by mold-replication technology which contained a structured polydimethylsiloxane (PDMS) surface with a standard array of 400 µm diameter pyramidal microwells. The PPCs differentiation was achieved from Royan hESC lines. HFP-MCs were isolated from 12-20 week human embryos. For aggregate formation, cell mixture suspension of hESC-PPCs and hFP-MCs was seeded on microwell chips. After aggregates generated by force aggregation and 24 hour incubation, were aspirated from the microwells and placed in static suspension culture.
Results: This platform produces approximately 4700 aggregates where each aggregate has a diameter of 70–120 µm, leading to uniformly sized, shaped and stable aggregates with low variation in diameter. We demonstrated that both cells are presented inside the aggregates after 48 hours of suspension culture and formed aggregates can be harvested easily without any additional forces, which prevent damage to aggregates during harvesting. Aggregates were expressing PP- and MC specific gene and protein markers after 48 hours culturing.
Conclusion: Altogether, pancreatic aggregates which are produced by our method formed round-shaped with well-defined borders aggregates and were able to be maintained stable in culture for short-range period in order to apply in *in vivo* transplantations and cell therapies.
Keywords: Microwell, Human Embryonic Stem Cell, Pancreatic Fetal Mesenchyme, Pancreatic Progenitor Cells, Organoid

Ps-38: Design and Construction of Expression Vectors for Evaluation of Mutual Interactions between Human Rax and E2f1 Transcription Factors

Ziaei Sh[1,2*], Shojaei P[2], Nasr-Esfahani MH[2], Dormiani K[2]

1. Department of Biology, Faculty of Science and Technology, ACECR Institute of Higher Education, Isfahan, Iran
2. Department of Molecular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: dormiani@yahoo.com

Objective: E2F1 transcription factor (TF) is a critical regulator of cell cycle and is required for G1 to S transition. Retina and anterior neural fold homeobox (RAX) play crucial roles in eye development and retinal progenitor cells (RPCs) specification. Because of the restricted rate of *in vitro* proliferation of RPCs, further studies to understand the molecular mechanisms involved in their maintenance are essential. In this study to investigate *in vitro* interactions between E2F1 and RAX, expression vectors harboring their coding sequences and promoters were designed and constructed. These vectors are co-transfected into 293T cells to analyze putative reciprocal interactions between these two TFs.
Materials and Methods: Based on in silico analysis and literature mining, several putative binding sites for E2F1 were predicted within 3258 bp upstream of the human RAX gene. Coding sequences of these two TFs and also E2F1 promoter region were amplified from the human genome and cloned into target expression vectors harboring mCherry and EGFP reporters respectively. Moreover, single transfection of these vectors into 293T cells by LTX lipofectamine was assayed microscopically.
Results: The Integrity of the expression vectors was examined by digestion and PCR. The target amplified sequences were also confirmed by sequencing analysis. Results indicated these regions were amplified without mutation, and successfully inserted into target vectors. Furthermore, transfection of these vectors into 293T cells confirmed the successful expression of these target genes and EGFP reporter driven by their putative promoter regions.
Conclusion: Considering successful construction and transfection of these vectors into 293T cells, *in vitro* evaluation of interactions between E2F1 and RAX, as critical modulators of proliferation in RPCs, might provide better insight into the mechanisms underlying retinal progenitor maintenance.
Keywords: E2F1, RAX, Retina Progenitor Cells, Proliferation

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