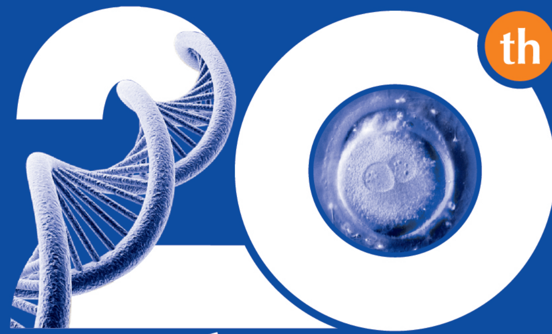


ROYAN

International Twin Congress

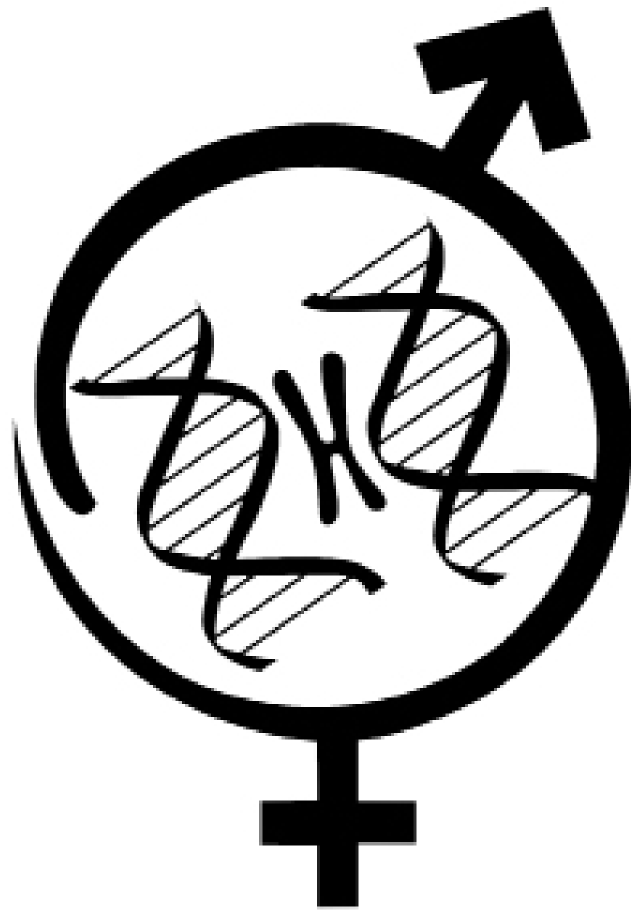
Reproductive Biomedicine & Stem Cells

15th Congress on Stem Cell Biology & Technology

Abstracts of

Royan International Twin Congress

15th Congress on Stem Cell Biology and Technology
28-30 August 2019



Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Contents:

● Collaborators 4
● Chairperson Wellcome Message 5
● Invited Speakers 6
● Oral Presentations 16
●Poster Presentations 18
● Authors Index 62

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Iranian Stem Cell Network
Iranian Council of Stem Cell Technology (ICST)
Tehran University
- **Science Division, Biology Faculty**

Cancer Research Institute, Bratislava, Slovakia
Eberhard Karls University Tübingen, Germany
- **Department of Traumatology Schnarrenbergstr**

Isfahan Medical University, Iran
- **Department of Genetic and Molecular Biology**

Ferdowsi University of Mashhad, Iran
- **Department of Biology**

Institute for Neurophysiology, Cologne, Germany
Kurdistan University of Medical Sciences, Sanandaj, Iran
- **Department of Anatomy, School of Medicine**

Max Planck institute for Molecular Biomedicine, Germany
- **Department of Cell & Developmental Biology**

Murdoch Children's Research Institute, Australia
- **Royal Children's Hospital**

Nagoya City University, Japan
National University of Ireland, Galway
- **Centre for Research in Medical Devices (CÚRAM)**

National Institute of Genetic Engineering and Biotechnology, Iran
National Institute of Immunology, India
San Raffaele Scientific Institute, Italy
- **Diabetes Research Institute (HSR-DRI)**

Shahid Beheshti University of Medical Science, Tehran, Iran
- **Research Center for Cellular and Molecular**

Tarbiat Modares University, Iran
- **Department of Biotechnology**
- **Department of Anatomy, Medical Science Faculty**
- **Department of Molecular Genetics**

Tehran University of Medical Sciences, Iran
- **Faculty of Biology**
- **Hematology, Oncology and Stem Cell Transplantation Research Center, Shariati Hospital**

The Rockefeller University , USA
- **Laboratory of Stem Cell Biology and Molecular Embryology**

TWICORE, Centre for Experimental and Clinical Infection Research, Germany
University of Cincinnati, College of Medicine, USA
- **Department of Molecular Genetics**

University of Manitoba, Canada
- **Basic Medical Science Building**

Congress Chairperson

Samaneh Hosseini

Dear Colleagues,

It is a great pleasure to cordially invite you to the **15th Royan International Congress on Stem Cell Biology and Technology**, in Tehran, the capital city of Iran. The ICSCBT 2019 is a 3-day gathering which will be held from August 28 to 30, 2019 at Razi International Conference Center, Tehran, Iran.

Following the organization of 14 successful events, the excellent program committee will offer a large number of invited lectures from renowned speakers all over the world which will ensuring that the 2019 conference will continue to build on the success of the 15th conference.

ICSCBT 2019 as a premier event for the dissemination of cutting edge research in stem cell and regenerative medicine; brings together distinguished and prominent researchers, scientists, clinicians, trainees, and students to discuss critical developments in basic and translational research and promote scientific information interchange. The meeting is led by keynote speakers each day and consists of plenary sessions, practical workshops and poster presentations.

The conference is organized by Royan Institute for Stem Cell Biology and Technology, the leading professional institute for stem cell research in Iran. The conference is held every year to make it an ideal platform for people to share views and experiences in stem cells, regenerative medicine and related areas.

We are honored to be able to host this important Congress in Tehran. Tehran and its environs are a place with spectacular natural beauty and abundant history and culture. Our multicultural city has many diverse neighborhoods of interest to visitors. We really appreciate it if you could join us in summer 2019 to both learn and share your latest results with professors, students, postdocs, and industrial researchers who make outstanding contributions to stem cell research. The ICSCBT 2019 will provide a perfect atmosphere for catching up with old friends and making new ones.

We wish you fruitful and enjoyable congress.

Samaneh Hosseini, Ph.D.
Congress Chairperson
15th Royan International Congress on
Stem Cell Biology & Technology

Invited Speakers

Is-1: Long Non-coding RNA ES 1 Controls The Proliferation of Breast Cancer Cells by Regulating the Oct4/Sox2/miR-302 Axis

Asadi H
Email: hossein_a1359@yahoo.com

Is-2: The Epigenetic Regulation and 3D Chromatin Organization in Embryonic Stem Cells

Atlasi Y

Department of Molecular Biology of The Radboud University, Nijmegen, The Netherlands
Email: Yaser.Atlasi@ncmls.ru.nl

Recent advances in chromatin biology have revealed a central role of transcriptional enhancers in regulating the spatiotemporal gene expression in mammalian cells. These regulatory elements interact with promoters and other enhancers to generate a complex and cell-specific network of chromatin interactions. This interaction network is crucial for proper regulation of gene expression and 3D genome organization. How enhancers are regulated during early embryonic development, however, is not well understood. In this presentation, I will focus on embryonic stem cells and discuss how the enhancer landscape is reprogrammed in different states of pluripotency. Using data obtained from different genome wide technologies, I will examine the mechanisms underlying enhancer activation and the extent to which enhancer-promoter rewiring contributes to spatiotemporal gene expression in different states of embryonic stem cells.

Is-3: The Translational Landscape of Ground State Pluripotency

Atlasi Y

Department of Molecular Biology of The Radboud University, Nijmegen, the Netherlands
Email: Yaser.Atlasi@ncmls.ru.nl

Translational control plays a central role in regulation of gene expression and can lead to significant divergence between mRNA- and protein-abundance. The translational landscape of early mammalian development and its impact on cellular proteome, however, remains largely un-explored. By measuring the mRNA-abundance, mRNA-translation rate and protein expression, I will discuss the global changes in gene expression and the relative contributions of different layers of gene regulation during the transition of naïve to primed state pluripotency.

Is-4: Elimination of Tumorigenic Pluripotent Stem Cells by A Small-Molecule Antibiotic

Bagheri R[1, 2], Pakzad M[1], Baharvand H[1, 2], Braun T[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, University of Science and Culture, Tehran, Iran
3. Max-Planck Institute for Heart and Lung Research, Department of Cardiac Development and Remodelling, Bad Nauheim, Germany
Email: sharif.moradi@gmail.com

Pluripotent stem cells (PSCs) exhibit unlimited self-renewal and the ability to differentiate into all cell types of the body including germ cells. Because of these key characteristics, PSCs hold great potential for regenerative medicine by providing therapeutically relevant cells for replacing the damaged or diseased tissues. Although these cells could be the next generation for cell therapy, the residual undifferentiated cells can give rise to teratomas, a form of benign tumor containing all the differentiated cell lineages. Therefore, it is of crucial importance to eliminate these remaining undifferentiated cells before injection into patients. So far, a broad array of approaches has been applied for eliminating these cells, among which small molecules appear to be a more effective strategy due to high safety, low cost, high metabolic stability, and easy delivery. Recently, the small-molecule antibiotic enoxacin was shown to have a prominent inhibitory effect on the viability of various cancer cells through enhancing the global maturation of microRNAs. Since (i) there is a high degree of molecular and behavioral similarity between cancer cells and PSCs and (ii) microRNAs play indispensable roles in PSC physiology, we hypothesized that it might be able to inhibit the viability and therefore the tumorigenicity of PSCs upon transplantation. Our results indicate that enoxacin considerably decreases the viability of human PSCs including human embryonic stem cells (ESCs) and human induced PSCs (iPSCs) as well as their clonogenicity and alkaline phosphatase activity. Furthermore, we find that enoxacin completely blocks *in vivo* teratoma formation capability of injected PSCs. Moreover, the expression level of mature microRNAs is significantly induced within 24 hours of PSC treatment with enoxacin. We also reveal that enoxacin exerts its inhibitory effects through stimulating the TARA-binding protein (TRBP), the physical partner of Dicer. Taken together, our data demonstrate that enoxacin could effectively remove residual undifferentiated PSCs, thereby maximizing the safety of PSC-based cell therapies.

Keywords: Pluripotent Stem Cells, Tumorigenicity, Enoxacin, MicroRNA, Cell Therapy

Is-5: Perturbation of Endogenous siRNA Level by DICER1 Overexpression in Adipose-Derived Mesenchymal Stromal Cells

Bidkhori HR[1, 2*], Farshchian M[1], Matin MM[3], Hasanzade H[1], Mirahmadi M[1], Dastpak M[1], Arghiani N, Bahrami M[1], Mehrzad S[1], Fozoni-Moghaddam B[1], Bahrami AR[1, 3], Rafatpanah H[2]

1. Stem Cells and Regenerative Medicine Research Department, ACECR-Khorasan Razavi, Mashhad, Iran
2. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Biology, Ferdowsi University of Mashhad, Mashhad, Iran
Email: hbidkhori@gmail.com

Background: The immunoregulatory characteristics of mesen-

chymal stromal/stem cells (MSCs) raise hope for treatment of inflammatory diseases. However, the immunosuppressive potential of MSCs is not always achieved and manipulating the interplay between MSCs and immune responses is recommended to enhance their function.
Despite exogenous sources for dsRNA transcripts derived from invading viruses, the vast majority of dsRNA has endogenous origin called endo-siRNA. Unlike invertebrate, mammalian cells lack a strong dsRNA processing machinery and molecular scissors such as DICER1 and RNAi pathway that could be detected in oocyte or embryonic stem cells. Meanwhile, differentiated cells elicit interferon secretion as a response to dsRNA. Furthermore, viral proteins with immunosuppressive functions has been widely used in different studies, here we used B18R, a cytokine inhibitor protein as a control in comparison with DICER1 induction state. We hypothesized that compensation of DICER1 expression in mammalian differentiated cells attenuate interferon response and anti-inflammatory cytokines.
Materials and Methods: We cloned GFP-2A-Puromycin fragment inframe in pCAGGS-hsDicer vector (Addgene Plasmid #41584) and pCDH513b (GFP) constructs were utilized for transfection in HEK293T and MSCs with Lipofectamin 2000. MSCs also were treated with 200 ng/µl B18R protein. Thereupon, dsRNA and interferon response genes were evaluated 48 hours post transfection using relative Real-Time PCR.
Results: Our data showed that DDX58 and RNase L were overexpressed in HEK293T and MSCs. Moreover, TNFAIP6 (TSG6) and OAS2 were down-regulated in HEK293T and IFIH1 was suppressed in MSCs. It is suggested that dsRNA recognition sensors (OAS2 and DDX58) and effector enzymes (RNase L) might be increased as a feedback, because of production of endo-siRNA following DICER1 expression. Consequently, Type I interferon and proinflammatory cytokines (IFIH1 and TSG6) significantly suppressed. In addition, we showed reduced levels of IL10 and INFβ after using B18R in MSCs which suppress the antiviral activity and interferon response.
Conclusion: It might be proposed that activation of RNA interfering pathway in MSCs paves the way for producing immunocompromised cells.

Is-6: Accelerating Orphan Drug Discovery Using Zebrafish Models of Rare Diseases

Crawford AD[1, 2, 3, 4]

1. Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Oslo, Norway
2. Tarbiat Modares University, Faculty of Agriculture, Tehran, and Faculty of Natural Resources & Marine Sciences, Noor, Iran
3. Institutes for Orphan Drug Discovery, Bremen, Germany
4. Theracule AS, Oslo, Norway
Email: alexander.dettmar.crawford@nmbu.no

There are approximately 7000 rare diseases, yet to date only a very limited number of orphan drugs are marketed. Developing new drugs requires significant funds and time, and in the case of rare diseases, the commercial return is often insufficient for the pharmaceutical industry. For many rare diseases, drug repurposing would represent a faster, cheaper and less risky option to identify drugs that would be of immediate therapeutic benefit to patients. A key obstacle currently in the way of a systematic evaluation of approved drugs for their potential to treat a large number of different rare diseases is the availability of suitable screening models to perform drug repurposing screens. Because 80% of rare diseases are genetic in origin, it is possible to create animal models that have similar genetic defects and that mimic the human pathological conditions. Zebrafish, with their high genetic, physiological and pharmacological similarity with humans, offer the possibility of performing rapid drug repurposing screens using microscale, *in vivo* models of human diseases. To date, we have generated zebrafish models for a number of rare neurodevelopmental disorders, including Dravet syndrome, Lennox-Gasteaux syndrome, Otahara syndrome, Batten disease, and Zellweger syndrome. Drug repurposing and drug discovery screens using some of these zebrafish models has led to the identification of both approved drugs and new drug candidates, one of which will enter a Phase 2a clinical trial in 2020.

Is-7: Translational Biodiscovery: Identifying and Developing New Drugs from Nature

Crawford AD[1, 2, 3]

1. Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Oslo, Norway
2. Tarbiat Modares University, Faculty of Agriculture, Tehran, and Faculty of Natural Resources & Marine Sciences, Noor, Iran
3. Institute for Arctic & Antarctic Biodiscovery, Medford, Oregon, USA
Email: alexander.dettmar.crawford@nmbu.no

Emerging challenges within the current drug discovery paradigm are prompting renewed interest in secondary metabolites as an attractive source of novel, structurally diverse small molecules that have been evolutionarily 'pre-selected' for bioactivity. With the recent validation of zebrafish as a biomedically relevant model for functional genomics and *in vivo* drug discovery, the zebrafish bioassay-guided identification of natural products is an attractive strategy to generate new lead compounds in a number of indication areas. We have recently developed a number of *in vivo*, microgram-scale, high-throughput bioassays based on zebrafish embryos and larvae for the systematic identification and pharmacological characterization of bioactive natural products. Zebrafish offer the ability to rapidly evaluate – at a very early stage in the drug discovery process – not only the therapeutic potential of natural products, but also their potential hepato-, cardio-, and neurotoxicities. Due to the requirement for only microgram quantities of compounds to be tested, *in vivo* assays based on zebrafish are useful not only for bioassay-guided isolation, but also for the subsequent derivatization of bioactive natural products prioritized for further development as drug discovery leads.

I-8: Interferon Beta: A Potential Candidate for The Treatment of Alzheimer's Disease

Dargahi L[*]**, Chavoshinezhad S**

Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: l_dargahi@yahoo.com

Interferon beta (IFNβ) is a cytokine with immunomodulatory properties, approved as the first treatment to modify the course and prognosis of the multiple sclerosis. IFNβ also possesses direct effects on the central nervous system, recently gained attention in the context of neuroinflammatory/neurodegenera-

tive diseases like experimental autoimmune encephalomyelitis, Parkinson's disease, stroke and spinal cord injury. Considering neuroinflammation, neural cell death and impaired neurogenesis as major players in Alzheimer's disease (AD), we examined the therapeutic potential of IFNβ in a rat model of AD. Since the brain bioavailbality of IFNβ is low in systemic routes of administration, we used intranasal (IN) approach which is shown to provide efficient delivery to the cortex and hippocampus and rescue the peripheral side effects. Our results showed that IN IFNβ treatment ameliorates spatial and passive avoidance learning and memory deficits induced by over-expression of mutant human APP gene in the hippocampus of adult rats. At cellular and molecular levels, IFNβ reduced APP expression, Aβ plaque formation, gliosis and pro-inflammatory responses as well as apoptosis in AD rat hippocampus. IFNβ also increased neurogenesis markers in the dentate gyrus neurogenic niche. Collectively, IN IFNβ can be a promising therapeutic approach to halt the disease pathology and improve cognitive performance in AD-like neurodegenerative context.

Keywords: Intranasal Interferon Beta; Alzheimer's Disease; Learning and Memory; Neuroinflammation; Neurogenesis; Apoptosis

Is-9:From Bench Science to Clinical Translation: Patents in Biomaterials.

D'Este M

AO Research Institute Davos,Clavadelerstrasse, Davos, Switzerland
Email: matteo.deste@aofoundation.org

Over the last few years, in many scientific disciplines there has been a strong accent on polarizing the research outcomes for the benefit of the society at large, rather than for restrict academic communities. In part, this is justified by the fact that most research is supported by taxpayers, but independently from economic aspects, the Science community is part of the society at large, and has the moral responsibility to contribute to the overall well-being and progress. This tendency is particularly strong in the biomedical field, where "clinical translation" has become a leitmotif. Intellectual property and patenting are key steps along the clinical translation path; however, researchers trained through a purely academic background sometimes have a blurry idea of these aspects or tend to neglect them. In this lecture, a few basic concepts on patenting will be introduced, including what a patent is and what a patent is not, and the difference between discovery, invention, and patentable invention will be illustrated. Typical mistakes to avoid throughout the process will be presented. The difference between drafting a scientific paper and drafting a patent will be highlighted, with a particular emphasis on the use of terminology. The steps between filing a patent and granting will be illustrated in the European framework. Finally, the process of patenting will be put in the wider context of Biomaterials Science and its clinical translation.

I-10: Y Chromosome Genes Play Roles in Sex-Dependent Development Outside of Sex Determination

Hosseini Salekdeh GH*, Taleahmad S, Meyfour A, Khani F, Nafian S, Alikhani M, Mollamohammadi S, Taee A, Hassani SN, Baharvand H

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

Despite the small number of Y chromosome genes, their adequate expression is required for regulation of transcription, translation, and protein stability of male individuals beyond sex-determination. In addition to their roles in male infertility, Y chromosome has an inevitable role in the sexual dimorphism of healthy and disease phenotypes and development. We previously reported that the Y chromosome genes, DDX3Y, is associated to neural induction in human embryonal carcinoma NTERA-2 cell line. Furthermore, TBL1Y may play an important role in cardiac development. We recently observed that another Y chromosome gene, KDM5D, may also contribute in cardiac differentiation of human embryonic stem cells (hESCs). We also found that most of the Y chromosome genes like SRY showed high expression level in primed versus naïve hESCs. Therefore, we hypothesize that SRY prevents WNT/β-catenin signaling by interaction and inhibition of β-catenin translocation. By the loss of function approach through depletion of SRY of the primed cells, we observed that the expression of pluripotency markers and WNT signaling target genes such as Brachuary, GCBM, TBX2 and TBX3 increased in si-SRY treated cells. In conclusion, we revealed that inhibition of SRY results in nucleus translocation of β-catenin and up-regulation of WNT signaling pathway which important to naïve conversion. Our findings present a potential role for the contribution of Y chromosome genes in sex-dependent development outside of sex determination.

I-11: Surface Immobilization of JAG-1 on PEG-based Microcapsules for Immunoprotection of Pancreatic Islets

Izadi ZH

Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: izadi.zhila@yahoo.com

Although transplantation of pancreatic islets is a promising approach for treatment of type 1 diabetes mellitus, the engraftment efficiency of these islets is limited by host immune responses. Extensive efforts have been made to immunoisolate these islets by introducing barriers on the islet surface. To date, these barriers have not successfully protected islets from attack by the immune system. In addition, the inevitable permeability of an islet capsule cannot prevent filtration by proinflammatory cytokines and islet self-antigens. Thus, we have developed a surface engineering approach for localized immonumodulation of the islet microenvironment. Jagged-1 (JAG-1), as a potent immunomodulatory factor, was immobilized on the islet surface by mediation of a double-layer of heterobifunctional poly (ethylene glycol) (PEG). Immobilization and functionality of JAG-1 on PEGylated islet surfaces were established. When cocultured with splenocytes, the JAG-1 conjugated islets induced a significant increase in regulatory T cells and regulated the cytokine levels produced by immune cells. The results demonstrated that JAG-1 immobilization could improve immunoprotection of pancreatic islets by localized modulation of the immune milieu from an inflammatory to an anti-inflammatory state. We also evaluated the effects of surface modification of these islets by JAG-1 in a xenotransplantation model. The

transplanted JAG-1/PEG/islets group showed a significantly reduced blood glucose levels compared with the control group of diabetic mice during the acute phase of the immune response to the transplanted islets. Our results demonstrated that surface modification has the potential to shift the immune system from an inflammatory to anti-inflammatory milieu and may offer a new prospective for immunoprotection of pancreatic islets.

Keywords: Type 1 diabetes; Immunomodulation; Surface immobilization; Jagged-1; Islet PEGylation

I-12: Candidate Non-Coding RNAs Regulate Cardiomyocyte Differentiation and Maturation Process

Kay M[1*], Mohammad Soltani B[1], Hosseini Aghdaei F[1], Ansari H[2], Baharvand H[2]

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, 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: maryam_kay2001@yahoo.com

WNT and TGFβ signaling pathways play critical regulatory roles in cardiomyocyte fate determination and differentiation. MiRNAs are also known to regulate different biological processes and signaling pathways. Here, we intended to find candidate miRNAs that are involved in cardiac differentiation through regulation of WNT and TGFβ signaling pathways. Bioinformatics analysis suggested hsa-miR-335-3p and hsa-miR-335-5p as regulators of cardiac differentiation. Then, qRT-PCR, dual luciferase, TOP/FOP flash, and western blot analyses were done to confirm the hypothesis. Human embryonic stem cells (hESCs) were differentiated into beating cardiomyocytes, and these miRNAs showed significant expression during the differentiation process. Gain and loss of function of miR-335-3p and miR-335-5p resulted in BRACHYURY, GATA4, and NKX2-5 (cardiac differentiation markers) expression alteration during the course of hESC cardiac differentiation. The overexpression of miR-335-3p and miR-335-5p also led to upregulation of CNX43 and TNNT2 expression, respectively. Our results suggest that this might be mediated through enhancement of WNT and TGFβ signaling pathways. Overall, we show that miR-335-3p/5p upregulates cardiac mesoderm (BRACHYURY) and cardiac progenitor cell (GATA4 and NKX2-5) markers, which are potentially mediated through activation of WNT and TGFβ signaling pathways. Our findings suggest miR-335-3p/5p to be considered as a regulator of the cardiac differentiation process.

Keywords: miRNA, Cardiomyocyte

Is-13: Personalised Cell Products from Gingival Stem Cells

Kosheleva NV[1, 2, 3*], Saburina IN[1, 3], Zurina IM[1, 3, 4], Gorkun AA[1, 3, 4], Eremin II[1], Pulin AA[1, 3, 5]

1. FSBSI Institute of General Pathology and Pathophysiology, Moscow, Russia
2. Lomonosov Moscow State University, Faculty of Biology, Moscow, Russia
3. Russian Medical Academy of Continuous Professional Education (FSBEI FPE) of The Ministry of Healthcare of Russia, Moscow, Russia
4. Sechenov First Moscow State Medical University, Institute for Regenerative Medicine, Moscow, Russia
5. FSBI "National Medical and Surgical Center named after N.I. Pirogov" of the Ministry of Healthcare of Russian Federation, Moscow, Russia
Email: n_kosheleva@mail.ru

One of the key problems of regenerative medicine are available autologous cell sources with huge differentiation potential for cell therapy. Mesenchymal stem cells (MSCs) are one of the sources of myoblasts during muscle tissue regeneration. Differentiation potential of MSCs is elevated under 3D culture conditions. The aim of this work was to study cell products for therapy of musculoskeletal dysfunction from human gingival MSCs (MSCs-g) cultured in 2D and 3D (spheroids) conditions. Biopsies of gingiva were collected from healthy donors (n=10) after their informed consent. Mucosa fragments were treated in collagenase type II (200 u/ml, 120min, 37oC, 5% CO_2). We used cultures at passages 3-4. Growth culture medium consisted of DMEM/F12 and 20% FBS (HyClone, USA), induction medium contained DMEM low glucose and 2% Horse Serum (BioInd, USA). Spheroids were formed on agarose plates with micro-wells made using 3D Petri Dishes (Microtissue, USA). Appearance of myotubes was monitored via live time-lapse microscopy in Cell-IQ device (CM Technologies, Finland). Secretome profile from 2D and 3D cultures was analyzed with 41plex kit (xMap technology, Millipore, USA). 2D and 3D cultures were fixed in 4% paraformaldehyde for analysis of MyoD and sarcomeric alfa-actin expression.
In 2D culture after induction and in 40% cases spontaneous myogenic differentiation took place. Multinucleated myotubes in 2D culture expressed only MyoD, a marker of early stages of myogenesis. Expression of sarcomeric alfa-actin, which is characteristic of differentiated muscle cells, was absent. In 3D culture differentiation was more effective. Differentiated spheroids did not contain early progenitor cells, but well-formed myofibrils with characteristic peripheral nuclei arrangement and cross-striation, marked by antibodies against sarcomeric alfa-actin. Moreover, level of proangiogenic factor VEGF in the media conditioned by spheroids significantly increased. On the model of calf muscle injury suspension of MSC-g only reduced size of scar, whereas spheroids promoted full organotypic recovery of muscle tissue.
3D culturing of MSCs-g in form of spheroids stimulates effective myogenic differentiation. Prevascularized microtissues from 3D cultures of accessible MSCs-g can find its place in personalized medicine for musculoskeletal dysfunction tissue replacement therapy *in vivo*. The study was financially supported by Russian Science Foundation (grant № 17-75-30066).

Keywords: Prevascularized Microtissue, 3D Culture, Spheroids, Mesenchymal Stem Cells, Gingiva, Muscle Regeneration

Is-14: Up-Regulation of A Male-Specific H3K4 Demethylase, KDM5D, Is Required for Cardiomyocyte Differentiation

Meyfour A[1, 2*], Pahlavan S[3], Ansari H[3], Baharvand H[3, 4*], Hosseini Salekdeh GH[2, 5, 6*]

1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Sci-

ence Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
5. Department of Systems and Synthetic Biology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran
6. Department of Molecular Science, Macquarie University, Sydney, NSW, Australia
Email: a.meyfour@gmail.com

Background: Y chromosome human proteome project (Y-HPP) has been implemented with a great mission to investigate the Y chromosome proteins expression and function, not only in sex determination and fertility, but also in organ development and organ-specific function. Previously, we showed that some Y chromosome genes are differentially expressed during cardiogenic differentiation of human embryonic stem cells (hESC). Among them, KDM5D along with its X-counterpart, KDM5C, were overexpressed during cardiac mesoderm stage.
Materials and Methods: The function of KDM5D in cadiomyocyte differentiation was assessed by knocking down of its expression using siRNA. At 48 hours after siRNA transfection, the siKDM5D transfected cells were analyzed for cell cycle progression. The expression of KDM5D, KDM5C and some of cardiac progenitor specific markers was assessed following KDM5D knockdown. Furthermore, the function of differentiated cardiomyocytes was studied by multielectrode array (MEA) system
Results: Although the expression level of KDM5C remained unchanged, down-regulation of KDM5D resulted in a substantial growth of differentiating cell population in S-phase of cell cycle while the expression level of cardiac progenitor specific markers diminished. Furthermore, no spontaneous beating was observed in KDM5D down-regulated cells while the beating was started in control cells on day 7 of cardiac induction.
Conclusion: Our findings show that KDM5D in co-operation with its X homologue as dose-sensitive genes are necessary for cardiomyocyte differentiation and provide further evidence on the importance of Y chromosome genes in cardiac development beyond sex determination.
Keywords: KDM5D, Y-HPP, Y chromosome, Cardiac Development

Is-15: Therapeutic Effects of BMSC-derived Exosome Packed with a miR-21-Sponge Construct in a Rat Model of Glioblastoma

Mowla SJ

Department of Molecular Genetics, Tarbiat Modares University, Tehran, Iran
Email: sjmowla@modares.ac.ir

Is-16: A Comparative Study of Tissue-Engineered Constructs from Acropora and Porites Coral in a Large Animal Bone Defect Model

Petite H*, Decambron A, Manassero M, Bensidhoum M, Lecuelle B, Logeart-Avramoglou D, Viateau V

1. Laboratory of Osteo-Articular Biology, Bioengineering and Bioimaging, University of Paris, Paris, France.
2. Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort Cedex, France
Email: herve.petite@univ-paris-diderot.fr

Background: To compare the therapeutic potential of tissue-engineered constructs (TECs) combining mesenchymal stem cells (MSCs) and coral granules from either Acropora or Porites to repair large bone defects.
Materials and Methods: Bone marrow-derived, autologous MSCs were seeded on Acropora or Porites coral granules in a perfusion bioreactor. Acropora-TECs (n = 7), Porites-TECs (n = 6) and bone autografts (n = 2) were then implanted into 25 mm long metatarsal diaphyseal defects in sheep. Bimonthly radiographic follow-up was completed until killing four months post-operatively. Explants were subsequently processed for microCT and histology to assess bone formation and coral bioresorption. Statistical analyses comprised Mann-Whitney, t-test and Kruskal-Wallis tests. Data were expressed as mean and standard deviation.
Results: A two-fold increase of newly formed bone volume was observed for Acropora-TECs when compared with Porites-TECs (14 sd 1089 mm3versus 782 sd 507 mm3; p = 0.09). Bone union was consistent with autograft (1960 sd 518 mm3). The kinetics of bioresorption and bioresorption rates at four months were different for Acropora-TECs and Porites-TECs (81% sd 5% versus 94% sd 6%; p = 0.04). In comparing the defects that healed with those that did not, we observed that, when major bioresorption of coral at two months occurs and a scaffold material bioresorption rate superior to 90% at four months is achieved, bone nonunion consistently occurred using coral-based TECs.
Discussion: Bone regeneration in critical-size defects could be obtained with full bioresorption of the scaffold using coral-based TECs in a large animal model. The superior performance of Acropora-TECs brings us closer to a clinical application, probably because of more suitable bioresorption kinetics. However, nonunion still occurred in nearly half of the bone defects

Is-17: Glucose Delivery System Based-Hydrogel Composite Scaffold for enhancing MSC survival

Denoeud C[1], Paquet J[1], Boisselier J[2], Becquart P[1], Moya A[1], Diallo A[1], Barbosa M[1], Marinesco S[3], Meiller A[3], Lefebvre E[2], Larreta-Garde V[2], Pauthe E[2], Potier E[2], Petite H[1]

1. Laboratory of Osteo-Articular Biology, Bioengineering and Bioimaging, University of Paris, Paris, France.
2. Biomaterial for Health Group, ERRMECe, Department of Biology, University of Cergy- Pontoise, France.
3. Neuroscience Research Center, AniRA-NeuroChem Platform, University of Lyon1, Lyon, France.
Email: herve.petite@univ-paris-diderot.fr

In the context of cell-based regenerative medicine, exogenously administered mesenchymal stromal cells (MSCs) exhibited a poor survival rate. A possible explanation for this limited cell survival is that, upon implantation, MSCs encounter a harsh ischemic microenvironment characterized by low oxygen tension and nutrient deprivation. This issue can be overcome by in situ supplying glucose that acts as the main metabolic fuel for MSCs in hypoxia and enhances their survival (Deschepper et al.2011 and 2013). The objective of the study is to engineer a tissue-construct that provides sufficient level of glucose to MSCs and enhances their survival when transplanted *in vivo*. To this aim, hydrogels containing fibrin, starch (a polymer of glucose) and AMG (an enzyme that re-

lease glucose from starch) were formulated. These injectable, self-supported hydrogels released glucose amounts in accordance with that required by hMSCs for their survival. *In vitro*, under near anoxia, MSCs loaded in fibrin/starch/AMG hydrogels exhibited a survival rate 115 times higher than the one loaded in fibrin hydrogels, after 14 days. Moreover, when ectopically implanted in nude mice, luciferase-labelled hMSCs loaded in fibrin/starch/AMG hydrogels exhibited a significant improvement of their viability (x4 after 14 days) in comparison to hMSCs loaded in fibrin gels as demonstrated by the follow-up of the luciferase activity by bioluminescence imaging. These data were further substantiated by monitoring the number of hMSCs remaining in the hydrogels implanted ectopically in mice. At day 14 days, fibrin / AMG / starch scaffolds contained 7.5 times more viable hMSCs than fibrin hydrogels. This work establishes for the first time that a construct based on a fibrin/starch/AMG hydrogel delivers glucose over time and enhances the survival of hMSCs. Most interestingly, the data obtained with hMSCs are now extended to adipose-derived MSCs and myoblasts.

Keywords: Mesenchymal Stem Cell, Cell Survival, Bone Repair.

Is-18: 3D Printing of a Hyaluronan Bioink With Double Gelation Mechanism for Independent Tuning of Shear-Thinning and Shape Fixation

Petta D[1, 2], Grijpma DW[2], Alini M[1], Eglin D[1], D'Este M[1*]

1. AO Research Institute Davos, Davos Platz, Switzerland.
2. Department of Biomaterials Science and Technology, University of Twente, Enschede, The Netherlands
Email: matteo.deste@aofoundation.org

Introduction: Biomedical 3D printing is emerging as an indispensable tool for the fabrication of tissue-like constructs. Extrusion-based bioprinting needs bioinks capable of low-shear extrusion, shape retention, adequate cell viability and composition similar to native tissues. In this study, we describe a tyramine-modified hyaluronic acid (THA) where the shear-thinning properties can be tuned independently of the final curing step owing to a double gelation mechanism. The relation between printability and rheological properties was determined, as well as their variations upon cell addition and medium modification.

Materials ans Methods: THA was synthesized by grafting tyramine to hyaluronan via amide formation in water achieving 15.5% substitution degree. THA was dissolved at final concentration of 2.5% w/v and added to 1) horseradish peroxidase and low concentration of H_2O_2 for enzymatic crosslinking, and 2) eosin Y (EO) for green light-triggered crosslinking. Rheological properties were measured with an Anton Paar MCR302 Rheometer. hMSCs from bone marrow; bovine chondrocytes from fetlock joints and hTERT fibroblasts were laden in the bioink. A bioprinter RegenHU 3D Discovery® was used to print over a range of nozzle geometries, pressures, and light exposures. Cell viability and its variation upon medium composition and printing conditions was determined with live/dead, CellTiter-Blue® and trypan blue assays in triplicates.

Results: The attributes of the THA bioink here presented are: i) single component but 2 distinct crosslinking mechanisms, allowing ii) tuning of shear-thinning independently of the post-printing curing; iii) no sacrificial components or rheological additives; iv) curing with visible light for shape stability; v) possibility to post functionalize; vi) preservation of hyaluronan structure owing to low modification degree. Enzymatic crosslinking of THA to a low controlled extent produced a soft shear-thinning gel with viscosity between 6 and 25 Pa•s at the shear rates experienced during extrusion, and therefore suitable for low-shear extrusion and consequently for cell encapsulation. Cell addition during enzymatic crosslinking revealed a dose-dependent decrease in viscoelasticity, however the effect was compensated by increasing the levels of H_2O_2 in the precursor preserving viability. Crosslinking with light did not produce this effect, and therefore the cell-induced decrease in viscoelasticity was putatively attributed to cell uptake of H_2O_2, even though at the ppm-range employed the H_2O_2 did not induce any cell death.

In PBS medium, EO displayed a dose-dependent cytotoxicity, likely due EO endocytosis. Cell death was significantly reduced at EO concentration below 0.02% and by adding 10% foetal bovine serum in the medium.

Cell-laden criss-cross constructs with high shape fidelity were printed, displaying 24 hours viability up to 93% for all cell types. Viability was maintained or increased after 14 days in culture.

Generally, printing fidelity and accuracy in biofabrication are still poorly defined and depend critically on the printer and the environmental conditions. We have identified the damping factor = G''/G' as measurable and reliable printability index, with the best results achieved with values between 0.4 and 0.6. The bioink was too fluid for values above and granular and poorly extrudable for values below this range. The damping factor is easily quantifiable, and therefore an absolute magnitude comparable objectively across laboratories to evaluate the printability of bioinks.

Conclusion: We have introduced a bioink based on hyaluronan with a double gelation mechanism for independent tuning of shear-thinning and final curing with visible light. These additional features allowed accurate tuning of the bioink properties, without additives. The damping factor was identified as reliable printability predictor.

Is-19: Aptamer Conjugated MSC-Derived Exosomes for Inducing Remyelination in Multiple Sclerosis Model

Ramezani M

Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
Email: RamezaniM@mums.ac.ir

Background: One of the well-known classes of secreted biological nanovesicles are exosomes, released by various cells. Some studies indicated that multiple sclerosis (MSC) secretome could be a capable therapeutic agent for the inflammatory and degenerative diseases such as MS and rheumatoid arthritis.

Materials and Methods: In the current study, we isolated and implemented the exosomes-derived MSC as a potent carrier to deliver therapeutic LJM-3064 aptamer. In this regard, the carboxylic acid-functionalized LJM-3064 aptamer was covalently conjugated to the amine groups on the exosome surface through EDC/NHS chemistry. Then the effects of MSC-derived exsosomes decorated with LJM-3064 aptamer (Exo-APT) was evaluated on remeylination processes and immunomodulatory activity in myelin oligodendrocyte glycoprotein (MOG35-55)-induced mouse MS model (EAE).

Results: Our results demonstrated that surface functionalization of exosomes with LJM-3064 aptamer produced synergistic immunomodulatory properties and remyelination effect.

In this regard, it was confirmed that prophylactic administration of Exo-APT significantly decreased the ameliorating disease severity by reducing Th1 response and increasing Treg population leading to the lowest inflammation and recruitment of inflammatory cells into the CNS. The obtained results were further supported by significant reduction in demyelination and pathological scores.
Conclusion: The prepared platform employing exosome-based nanomedicine as a novel approach for managing MS would hopefully pave the way to introduce a versatile approach toward an effective clinical reality.
Keywords: Multiple Sclerosis (MS), Mesenchymal Stem Cell, Exosome, Aptamer

Is-20: Importance of Preclinical Imaging for Performing Cutting-Edge Research

Solgi R[1, 2, 3], Mojabi ZS[1, 2], Tanha K[1, 2], Nosrati A[1, 6], Ghadiri H[1, 2, 3], Ay MR[1, 2, 3], Sharif-Paghaleh E[1, 3]

1. TUMS Preclinical Core Facility, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Medical Physics and Biomedical Engineering, TUMS Preclinical Core Facility, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Medical Physics and Biomedical Engineering, Research Center for Molecular and Cellular Imaging, TUMS Preclinical Core Facility, Tehran University of Medical Sciences, Tehran, Iran.
4. Department of Immunology of Faculty of Medicine, TUMS Preclinical Core Facility, Tehran University of medical sciences, Tehran, Iran.
5. Department of Immunology of Faculty of Medicine, Faculty Member of Division of Imaging Sciences and Biomedical Engineering, Faculty of Life Sciences and Medicine, King's College London, TUMS Preclinical Core Facility, Tehran University of medical sciences, Tehran, Iran.
6. Department of Chemistry, Shahid Beheshti University (SBU), Tehran, Iran.
Email: e.sharif@tpcf.ir

Background: The importance of preclinical research in the field of stem cell and rehabilitation medicine is a known fact. This is mostly demonstrated in development of novel stem cell therapies as well as understanding more about the stem cell itself. In more detail, during drug (in this case stem cells) development, preclinical testing is vital which includes monitoring, bio-distribution and pharmacological studies. We at TPCF are seeking to strive and work for bettering the preclinical studies in Iran.
Materials and Methods: Cutting-edge technology and Imaging modalities are one of the most important tools for performing informative and innovative preclinical studies. We also at TPCF are equipped with high technology imaging modalities, all of which are designed and manufactured by the TUMS knowledge-based companies. Moreover, TPCF is connected to the country's laboratory network so that researchers can use the lab's services at a lower cost.
Currently, TPCF is composed of the following facilities:
- Nuclear Medicine Services:
• Micro-PET Imaging Laboratory
• Micro-SPECT Imaging Laboratory
• Handheld Gamma Camera Imaging Laboratory
- Optical Imaging Services:
• Optical Planar Imaging Laboratory
• Optical Tomography Imaging Laboratory
- Micro-CT Imaging Laboratory
- Micro- Ultrasound Imaging Laboratory
- Image Processing & Analysis Laboratory
- Chemistry Laboratory
- Radiochemistry Laboratory
- Histology Laboratory
- Molecular Biology Laboratory
- Tissue Culture Laboratory
- Small Animal Surgery Section
- Small Animal Holding

Results:
Key features of TPCF:
• There are roughly around 20 similar facilities in the whole world.
• We are the only preclinical core facility in the entire Middle East.
• In terms of the variety of imaging devices and lab services, TPCF is among the top 10 core facilities in the world.
• All devices are made by Iran.
• Builders are all professors of Tehran University of Medical Sciences.
• Key services of TPCF:
• *In vivo* Image acquisition services using the above imaging modalities
• Image analysis services
• Educating scientists, clinicians and pharma on the available cutting-edge technology through 3 level workshops, seminars and journal club
• *In vitro* services: chemistry, biology, cell and tissue studies with focus on radioactive experiments
• Animal care and surgery services
Conclusion: Stem cell and cell therapy are thought to be the future of Medicine. A straight forward but important question for any cell therapy is: Where do they go? We at TPCF have established a facility where we could address this question and could lead us to identify how and why they go to a specific location. Gaining insight on these could then improve the efficacy of cell and stem cell therapies. For more information please visit us on www.TPCF.ir
Key words: Multimodal Imaging, Preclinical Studies, Radiation.

I-21: Milestones in the Development of Bioprosthetic Materials Based on the Mammalian Pericardium Tissue

Grebenik EA[1], Lazhko AE[2], Timashev PS[1, 3]

1. Institute for Regenerative Medicine, Sechenov First Moscow State Medical University, Moscow, Russia
2. Institute of Photonic Technologies, Research Center, Crystallography and Photonics, Moscow, Russia
3. N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia
4. Kurnakov Institute of General and Inorganic Chemistry of Russian Academy of Sciences, Moscow, Russia
Email: timashev.peter@gmail.com

The extensive research on the functional and structural properties of mammalian pericardium transplants has become a basis for their wide use in different fields of reconstructive surgery. The transplants manifested themselves in the fabrication of prostheses (cardiac valves, blood vessels, etc.) and for their direct application as membranes in dentistry, closure of various cavities and other purposes. To eliminate the immunogenicity and increase proteolytic stability, a number of

techniques have been developed based on decellularization and crosslinking. As a crosslinker, GA is used most frequently. However, less toxic functional analogues (genipin, epoxy-compound, isocyanates, carbodiimides, etc.) may potentially replace GA in the fabrication of pericardium tissue-based bioplastic materials. In our study, we performed a comprehensive characterization of decellularized bovine pericardium cross-linked via different chemical mechanisms. Their structure, biodegradability, biomechanics and cytotoxicity were compared to that of non-crosslinked matrices pointing out individual advantages and drawbacks. *In vivo* study was performed with a clinically approved crosslinker, epoxy-compound, based on multiparametric bioimaging. We revealed the progression of hypoxia and severe calcification of the crosslinked implant in subcutaneous murine model. To overcome the biomechanics-associated side effects, we employed the processing of the samples in supercritical carbon dioxide medium. This allowed to reduce the stiffness without compromising cytotoxicity and biodegradability.

The research was supported by the Russian Science Foundation under Grant No. 18-15-00401 (*in vitro* and *in vivo* studies) and Russian Foundation for Basic Research under Grant No. 18-33-00982 mol_a (supercritical carbon dioxide treatment).

I-22: Creation of Robust *In Vitro* Models to Study Liver Disease

Boon R[1], Tricot T[1], Kumar M[1], Topraksihar B[1], Deboeck J[1], De Smedt J[1], Ghosh S[1], Helsen N[1], Hu W[2], Neyts J[3], Grunsven LV[4], Verfaillie C[1]

1. KU Leuven Stem Cell Institute, Belgium
2. University of Minnesota, Minnesota, USA
3. Rega Institute Leuven, Leuven, Belgium
4. Vrije Universiteit Brussel, Brussel, Belgium
Email: catherine.verfaillie@kuleuven.be

We have developed *in vitro* models to study liver disease, such as liver inflammation and fibrosis, as seen in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH); or hepatitis viral infection; or to enhance our ability to detect drugs that cause acute or repeat dose drug induced liver injury (DILI) assessment, and this in medium to high throughput format. This requires that the model consist at least of (i) longer-term stable functioning hepatocytes that can be damaged by a compound /insult; resident macrophages (KCs); and hepatic stellate cells (HSCs) that can respond to this damage. In addition, the cells would ideally also contain stress reporter genes to allow high-content image-based definition of cell stress. Finally, the model should be down-scalable to 96 (or 384) well format allowing drug screening. Data will be presented describing studies towards this goal starting from induced pluripotent stem cells.

I-23: Use of iPSC Derived Hepatocytes for Liver Generation/Replacement

Tricot T[1], Kumar M[1], Topraksihar B[1], Deboeck J[1], Boon R[1], Roskams T[2], Meuleman Ph[3], Ross J, Verfaillie C[1]

1. KU Leuven Stem Cell Institute, Leuven, Belgium
2. Department of Pathology, University of Leuven, Leuven, Belgium
3. Deaprtment Diagnostic Sciences, UGent, Leuven, Belgium
4. Miroatrix, Edina, MN, USA
Email: catherine.verfaillie@kuleuven.be

Terminal liver failure caused by acute liver toxicants or by cirrhosis caused by alcohol, non-alcoholic steatohepatitis or viral infections, can only be treated with liver transplantation. However, due to the short supply of organs, many patients cannot be transplanted. Therefore, other sources of cells are being evaluated, including hepatocytes from pluripotent stem cells (PSCs). Data will be presented demonstrating that hepatocytes (and endothelial cells) derived from PSCs can be contemplated for grafting *in vivo* in the setting of liver failure, or can be used to recreate liver tissue *in vitro* by recellularization of decellularized liver scaffolds.

I-24: Use of iPSC Derived Brain Cells to Model Neurodegenerative Disorders

Garcia Leon J[1], Claes Ch[1], Daele JVD[1], Furtado R[1], Neyrink K[1], Nami A[1], Damme PhV[2], Bosch LVD[2], De Strooper B[2], Verfaillie C[1]

1. KU Leuven Stem Cell Institute, Belgium
2. VIB-KU Leuven Center for Brain & Disease Research, Belgium
Email: catherine.verfaillie@kuleuven.be

Despite major advances in our understanding of neurodegenerative disorders, no efficient therapies are available for patients with dementia, motor neuron disease and other neurodegenerative disorders. With the advent of pluripotent stem cells (PSCs) it now becomes possible to better model human disease *in vitro* (and in humanized mice), which may lead to the development of novel therapies for these currently untreatable disorders. Data will be presented on how to build such models, and how to use PSC-derived cells to identify novel therapeutic targets and therapies.

Is-25: Interactions between Surface Nanopatterns, Cells, and Bacteria

Zadpoor AA

Department of Biomechanical Engineering, Delft University of Technology (TU Delft), Delft, The Netherlands
Email: a.a.zadpoor@tudelft.nl

During the last decade, the importance of physical cues in regulating cell response has been discovered. The physical cues may include the stiffness of the biomaterial within which the cells are residing as well as the nanopatterns they are exposed to and the applied forces (stretching/contraction). In the vast majority of the cases, the mechanisms underlying such effects are mechanobiological in nature. In this talk, I will discuss the importance of surface nanopatterns, as one of the major types of physical cues, for determining the differentiation of stem cell towards the osteogenic lineage and preventing implant-associated infections through the creation of surfaces with bactericidal effects. The fabrication, characterization, and biological assessment of the nanopatterns will receive the most attention. In addition to surface nanopatterns, the other types of surface treatments that could be used for achieving both above-mentioned goals will be also covered.

Is-26: Meta-Biomaterials: A New Generation of Biomaterials with Impossible Properties

Zadpoor AA

Department of Biomechanical Engineering, Delft University of Technology (TU Delft), Delft, The Netherlands
Email: a.a.zadpoor@tudelft.nl

Architected materials with properties that were once considered impossible are being currently developed by a wide range of researchers from different disciplines. Meta-biomaterials are a new addition to these novel classes of metamaterials, where otherwise impossible combinations of mechanical, mass transport, and biological properties are created through complex geometrical designs as well as complex spatial distributions of material properties at multiple scales starting from macroscale and going all the way down to nanoscale. This allows for incorporation of multiple favorable functionalities into one single piece of biomaterial including improved tissue regeneration performance and minimized risk of implant-associated infections. Progress in 3D and 4D printing, origami, nanofabrication, and self-folding is at the center of these developments, as the development of meta-biomaterials requires application of multi-scale and multi-material fabrication and activation techniques. This talk will introduce the concept of meta-biomaterials and will present an overview of the meta-biomaterials developed in my lab during the past decade.

Oral Presentations

Os-1: Single-Cell Transcriptome of Dugesia Japonica

Bagherzadeh R[1, 2, 3, 4*], Alipanah H[5], Sharifi-Zarchi A[1,5], Gusev O[6, 7], Baharvand H[1, 3], Agata Kiyokazu A[2, 4]

1. Department of Computer Engineering, Sharif University of Technology, Tehran, Iran
2. Division of Genomic Technologies, RIKEN Center for Life Science Technologies (CLST), Yokohama, Japan
3. Department of Biophysics, Kyoto University, Kyoto, Japan
4. Extreme Biology Laboratory, Institute of Fundamental Medicine and Biology, Kazan, Russian Federation
5. Department of Life Sciences, Gakushuin University, Tokyo, Japan
6. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
7. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: rezaBagherzadeh.email@gmail.com

Background: The planaria Dugesia japonica as an *in vivo* model for stem cell and regenerative biology is a flatworm contains a large population of pluripotent stem cells -called neoblasts - which enable the remarkable regeneration capacity characteristic of the planarian.
Materials and Methods: For the first time, we have profiled thousands of single cells in Dugesia japonica using 10x Genomics Chromium Single Cell 3' Solution in order to characterize the different cell populations of the adult worms and understand the gene expression changes responsible for neoblast that maintain the homeostasis by continuously differentiating to all adult cell types. Therefore, single cell transcriptome profiling of adults should reveal mature and progenitor cells.
Results: Computational analysis of single-cell transcriptomes has allowed us to identify various Head abundant specific cell types, neoblast subpopulations plus major differentiated cell types and subpopulations. Our in silico prediction of future cell states from the transcriptional changes and lineage reconstruction method placed all available major cell types onto single lineage trees that connects all cells to specific cell groups and provides insights into single-cell trajectories. We further elucidate the contributions of each cell type in the whole organism/ specific tissues using cell type-specific gene expression references of our scRNA-Seq toward deconvolving of available bulk RNA-Seq.
Conclusion: Taken together, our results have extensively characterized the different cell types in adult planaria, Dugesia japonica, and demonstrate the importance of single-cell transcriptome analysis for mapping and reconstructing fundamental processes of developmental and regenerative biology.
Keywords: Single-Cell Transcriptome, Dugesia Japonica, Planaria, Stem Cell and Regenerative Biology, 10X Chromium

Os-2: *In Vitro* Recapitulation of Cardiac Developmental Microenvironment for Biomimetic Maturation of Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Kermani F[*], King O, Terracciano C

National Heart and Lung Institute, Imperial College London, London, United Kingdom
Email: kermani_fatemeh@yahoo.com

Background: Cardiovascular diseases are a leading cause of death worldwide which mostly result in cardiomyocytes (CM) loss. CM death during myocardial infarction and heart failure causes clinical challenges due to the heart inability to self-repair. Hence, the advent of human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) which can truly differentiate into CM is a promising tool for clinical and research applications. hPSC-CM, however, show neonatal-like phenotype which structurally and functionally differs from adult CM. Several maturation strategies have been applied to encourage the adult-like phenotype in CM and it is thought that recapitulating the physiological microenvironment for native CM allows them to fully mature. In this project, we combined three maturation approaches, including the use of a three-dimensional (3D) platform, time in culture and co-culturing with endothelial cells. We aimed to investigate if co-culture and time in culture influence human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) calcium (Ca^{2+}) cycling and metabolic function to better resemble adult-like CM.
Materials and Methods: We co-cultured early (day 17-20) and late (day 45-52) hiPSC-CM with human umbilical vein endothelial cells (HUVEC) in 3D fibrin hydrogels for 7 days. Immunostaining, mitochondrial membrane potential assay, Seahorse assay and optical mapping were performed to investigate whether co-culture with HUVEC and/or time in culture altered hiPSC-CM functional properties.
Results: We observed that the hiPSC-CM spontaneous beating rate was significantly reduced in co-cultures regardless of age. Furthermore, HUVEC significantly decreased hiPSC-CM mitochondrial membrane potential while time in culture had the opposite effect. There was not significant difference in the oxygen consumption rate among different conditions. Finally, our data showed that HUVEC prolonged hiPSC-CM Ca^{2+} transients in late co-cultures.
Conclusion: In conclusion, our data indicate that the combination of the above-mentioned maturation strategies manipulate hiPSC-CM mitochondrial and Ca^{2+} handling properties; however, this does not enable them to completely resemble the adult-like phenotype and other factors need to be also included to render these cells more suitable for heart regeneration strategies.
Keywords: Human Induced Pluripotent Stem Cell, Maturation, Three-Dimensional Culture, Time in Culture, Co-Culture

Os-3: Chondrogenic Related Genes Expression on IGF1-Induced Synovial Membrane Mesenchymal Stem Cells Isolated from Osteoarthritis Patients

Marlina M[1*], Rahmadian R[1], Yanti Ni Luh[2], Munshy Ubaydillah [2], Kusuma H[2], Satrio W[2], Rizal R[2], Amalia A[2], Widodo W[2], Wahyu W[3]

1. Biomolecular and Biomedical Research Centre, Aretha Medika Utama, Bandung, Indonesia
2. Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
3. Faculty of Pharmacy, Andalas University, Padang, Indonesia
Email: marlina_adly@yahoo.com

Background: Osteoarthritis (OA) is the most common joint disease that affect more aged-people and decline the quality of life. Autologous chondrocytes implantation (ACI) is current popular cell-based treatment for OA patients. However, this therapy has several disadvantages due to the involvement of surgery procedure. Mesenchymal Stem Cells (MSCs) has capability to differentiate become several cell types including adipocytes, chondrocytes and osteocytes. Enhancement of MSCs differentiation can be the alternative approach for cell-based therapy. IGF-1 is one of growth factor that regulate the chondrogenic differentiation. This study is aimed to evaluate the differentiation potency of MSCs derived from synovial membrane of OA patients (SMMSCs) with various concentration of IGF-1 (0, 75, 150, 300 ng/mL) as inducer

Materials and Methods: The evaluation of SMMSCs differentiation was performed by chondrogenic markers expression such as SOX9, ACAN, and COL2 by real time PCR analysis.

Results: The concentration 150 ng/mL of IGF-1 produced the highest level of all gene expressions among the group treatments both in a week and two weeks of treatment. It was shown a significant increase of the expression levels during two weeks of treatment. Aggrecan is shown to have the highest level of expression among the other markers which means the change in ECM matrix during chondrogenesis is already started from the second week.

Conclusion: From this study, 150 ng/mL of IGF-1 is the best concentration for inducing chondrogenic differentiation.

Keywords: Synovial Membrane Mesenchymal Stem Cells, Chondrogenesis, IGF-1,SOX9,COL2

Os-4: Design, Construction and Functional Evaluation of a Polycistronic Episomal Vector with The Aim of Human iPS Cell Induction

Mmahdavi B[1, 2*], Rezaei N[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

Background: Stem cell biology and personalized regenerative medicine are enormously influenced by induced pluripotent stem cells (iPSCs). Several approaches are accomplished for iPSCs induction. Amongst, nonviral episomal vectors provide a relatively easy, low-cost and low-risk way of introducing reprogramming factors into the somatic cells. However, compared to integrated approaches, the efficiency of this method is very low. In the current study, we have constructed a polycistronic extrachromosomal plasmid containing Woodchuck Hepatitis Virus Posttranscriptional Regulatory (WPRE) element, which improves the efficiency of iPSCs generation.

Materials and Methods: We developed a polycistronic plasmid containing a single expression cassette with four human pluripotency transcription factors (LIN28, NANOG, SOX2, and OCT4) along with the EGFP reporter gene through using a 2A peptide sequence. WPRE was cloned in downstream of described polycistronic fragment, in a separate construct. These two plasmids were transfected into target cells and EGFP expression was assessed at defined time points by fluorescent microscopy and flow cytometry. The mRNA and Proteins Expression Levels were determined by RT-qPCR and Western blotting.

Results: Analysis of transfected cells showed that the expression level of proteins significantly increased in the presence of WPRE, which in turn gave rise to improvement in the efficiency of iPSCs induction. Also, a similar amount of reprogramming factor's expression was evaluated by Western blot assay demonstrated an equivalent stoichiometric expression of 2a-mediated factors.

Conclusion: We developed a stabilized extrachromosomal polycistronic plasmid as a simple and feasible tool for efficient iPSCs induction. This relatively small vector showed concomitant, high-level expression of the four reprogramming factors with similar titers, which are considered as the critical parameters for efficient and consistent reprogramming. Moreover, Utilized WPRE element in the expression cassette increased the mRNA stability and consequently the yield of protein expression in the target cell.

Keywords: iPSCs, Extrachromosomal Plasmid, Polycistronic, 2A Peptide, WPRE Element

Os-5: Assess The Viability and Intracranial Biodistribution of Human Embryonic Stem Cell-Derived Dopaminergic Progenitors in Rats Using Non-Invasive Imaging Techniques

Mousavinejad M*, Hill CH, Taylor A, Murray P

Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Nuffield Building, Crown Street, Liverpool, L69 3BX UK, Liverpool, United Kingdom
Email: masoum@liverpool.ac.uk

Background: Human embryonic stem cells (hESCs) and induced pluripotent stem cells have great potential for modelling and treating neurodegenerative diseases as they are able to differentiate into neuronal and glial cell linages in response to appropriate signals. Parkinson's disease (PD) is one of the most common neurodegenerative disorders affecting older people and is characterized by degeneration of dopaminergic neurons in the substantia nigra, leading to tremor, muscle rigidity and instability in movement. The ineffectiveness of current medications for PD has motivated the development of therapies based on hESC-derived ventral midbrain (VM) progenitors which have the potential to replace the neurons lost due to the disease, thereby restoring the dopamine levels in the patient's brain. A current challenge, however, is to track the transplanted VM progenitors and determine their contribution to functional recovery. Therefore, the first aim of this study was to implement a non-invasive imaging strategy that would allow the behaviour of hESC-derived VM progenitors to be monitored non-invasively following stereotactic injection into the rat brain. The second aim was to determine if the labelling techniques used to visualise the cells in vivo had any effect on the tumourigenicity, differentiation potential or immunogenicity of the injected VM progenitors.

Materials and Methods: To visualise the cells, we used a bimodal imaging approach that allows the viability and tumourigenic potential of luciferase-expressing hESC-derived VM cells to be monitored with bioluminescence imaging (BLI), and the intracranial biodistribution of VM progenitors labelled with fluorescent iron oxide particles (IOPs) to be assessed with magnetic resonance imaging (MRI). To assess the differentiation potential and immunogenicity of the VM progenitors, at the end of the experiment, histological sections were analysed to assess the effect of the labelling strategies on VM differentiation (tyrosine hydroxylase expression), and immunogenicity (staining

for GFAP, a marker of activated astrocytes).
Results: The preliminarily results showed that the engrafted-VM progenitors formed neurons that expressed the dopaminergic neuron-specific marker, tyrosine hydroxylase (TH), suggesting that the labelling techniques did not negatively impact on the ability of the VM progenitors to mature and differentiate into TH+ neurons in vivo. Moreover, BLI and MRI showed no evidence for tumour formation post implantation, suggesting that the progenitors are not tumourigenic, irrespective of whether they express a genetic reporter, or are labelled with IOPs. MRI showed that the cells remained within the striatum and did not migrate to other brain regions.
Conclusion: Histological analysis showed that the labelling techniques did not affect the ability of the VM progenitors to differentiate. However, we observed that the presence of IOPs, but not luciferase, increased the extent by which GFAP+ host astrocytes accumulated around the hESC-derived cells.
Keywords: Stem Cells, Parkinson, Bioluminescence, Magnetic Resonance Imaging

Os-6: Histone Deacetylase 3 as An Orchestrator of Pancreatic Acinar Cell Regeneration and Neoplasia

Movahed M[1*], Stedman A[1], Dirami T[1], Quilichini E[1], Montgomery R[2], Bassel-Duby R[3], Olson E[3], Sonda S[4], Haumaitre C[1]

1. National Center of Scientific Research (CNRS), National Institute of Health and Medical Research (INSERM), Paris, France
2. Department of Molecular Biology, Hamon Center for Regenerative Science and Medicine, Dallas, United States
3. College of Health and Medicine, Tasmania, Australia
4. miRagen Therapeutics, Inc, United States
Email: mandana.movahed@etu.upmc.fr

Background: Introduction 95% of pancreatic cancer originates from exocrine acinar and ductal cells. In response to insults, such as inflammation during pancreatitis, acinar cells are able to regenerate, by undergoing transient acinar-to-ductal metaplasia (ADM). However, this cellular plasticity can also facilitate pancreatic neoplasia and constitutes a risk factor for pancreatic cancer (PDAC). Objective Our objective was to uncover new regulators of pancreatic regeneration and neoplasia. We previously highlighted roles of HDAC epigenetic modifiers in pancreas development and disease. Here, we focused on Histone deacetylase 3 (Hdac3), providing its enzymatic activity to the NCoR/SMRT transcriptional complex, and can act both as an activator and repressor of transcription.
Materials and Methods: We conditionally inactivated Hdac3 in mice, either in pancreatic progenitors using Pdx1-Cre; Hdac3fl/fl mice or in pancreatic acinar cells with Ptf1a-CreER; Hdac3fl/fl mice. Pancreatitis was induced using cerulein treatment.
Results: We found that lack of Hdac3 in pancreatic progenitors leads to a strong pancreatic hypoplasia and early lethality in mice with loss of acinar cells and ADM. While Hdac3 inactivation in pancreatic acinar cells perinatally and in adults has no effect at homeostasis, it severely impairs pancreatic regeneration and induces neoplasia in the context of acute pancreatitis.
Conclusion: Our results show that Hdac3 is specifically involved in acinar cell regeneration, recapitulating an embryonic program. Elucidating the specialized functions of HDAC3 in acinar regeneration and neoplasia initiation is crucial to exploit this epigenetic factor as a biomarker and identify new regulators and therapeutic targets of pancreatitis and pancreatic cancer drivers.
Keywords: Regeneration, Acinar Cells, HDAC3

Os-7: Breast Cancer Subtypes of Indonesian Patients based on Cd44/Cd24 Expression and Immunohistochemical Analysis

Widowati W[1*], Jasaputra D[1], Heriady Y[2], Faried A[3], Rizal R[4], Widodo W[4], Wibowo S[4], Girsang E[5], Lister I[5]

1. Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
2. Faculty of Medicine, Bandung Islamic University, Bandung, Indonesia
3. Faculty of Medicine, University of Prima Indonesia, Medan, Indonesia
4. Department of Neurosurgery and Stem Cell Working Group, Faculty of Medicine, University of Padjadjaran, Dr. Hasan Sadikin Hospital, Bandung, Indonesia
5. Bimolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia
Email: wahyu_w60@yahoo.com

Background: This research was aimed to define characteristic of breast cancer subtypes based on CD44/CD24 expression using flow cytometry analysis compared to ER, PR, HER2, Ki67, and P53 expression through immunohistochemistry assay.
Materials and Methods: Breast cancer cells were isolated through enzymatic methods using proportional amount of Collagenase Type 1, Hyaluronidase, and Trypsin from BC tissue from 3 different patients. Flow cytometry analysis was performed to analyze the expression of CD44/CD24 markers in isolated breast cancer cells and Hs587t, SK-BR-3, MDA-MB 468, ZR-35-70-1 and MCF-7 cell lines used as comparative cells. Immunohistochemical analysis was conducted to examine the expression of ER, PR, HER2, Ki67, and P53 in BC tissue sections.
Results: We successfully isolated breast cancer cells from 3 different patients through enzymatic method. All isolated BC showed fibroblast cell-like morphology. Immunohistochemical analysis showed that BC patient 1, 2, and 3 classified as Luminal A, Normal breast-like fibroadenoma, and Luminal A subtype respectively. Isolated BC cells showed high proportion of CD44+/CD24- subpopulation (>90% in patients 1 and 2; >60% in patient 2). CD44+/CD24- has been reported to have progenitor/stem cells characteristics. However Luminal A and Normal breast-like fibroadenoma have the most favorable prognosis, the cell isolated from both subtypes contain CD44+/CD24- subpopulation.
Conclusion: The isolated BC cells from 3 different patients with different cancer subtype showed different pattern of CD44+/CD24- expression. The highest subpopulation of isolated cells was CD44+/CD24-. These both different BC cell subtypes can be used as cell model for BC targeted therapy.
Keywords: Breast Cancer,CD44+,CD24-,Cell Line

Poster Presentations

Ps-1: A Holistic View to Cisplatin-Resistant Ovarian Cancer Determined Proteins and Signaling Pathways Related to Cancer Cell Polyploidy

Adibi Sedeh R[1*], Moein Sh[1], Gheisari Y[1, 2]

1. Department of Genetics and Molecular Biology, Isfahan University of Medical Sciences, Isfahan, Iran
2. Regenerative Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
Email: ygheisari@med.mui.ac.ir

Background: Ovarian carcinoma is the fifth cause of death from cancer in women with a high proportion of chemotherapy resistance and recurrence in patients . A survey on the reasons for tumor recurrence demonstrates that cancer therapeutic approaches are one of the suspects. Cisplatin is the first line of treatment in ovarian cancer but various clinical reports show a high rate of recurrence post-cancer treatment with this drug. It is supposed that cisplatin with its microtubule-destabilizing role leads to the formation of polyploid cells that are capable of producing malignant cancer stem cells. With a focus on the main regulatory pathways of cell polyploidy and depolyploidy, we re-analyzed a microarray dataset of cisplatin treatment on ovarian cancer cell line to investigate the main accelerators of tumor relapse.
Materials and Methods: GSE58470 mRNA microarray dataset deposited by Arrighetti et al was downloaded from the gene expression omnibus (GEO) database. The quality of microarray data was measured by principal component analysis using the ggplot2 package of R. Using GEO2R tool of GEO, genes with adjusted P-value≤0.05 were assumed as differentially expressed (DE). Using CluePedia plugin version 3.7.1 of Cytoscape, a protein-protein interaction network was constructed and topology of network analyzed by Cytoscape Network Analyzer tool and nodes with the highest degree centrality parameters were identified. Pathway enrichment analysis was performed using ingenuity pathway analysis (IPA) and signaling pathways with P-value≤0.05 were determined.
Results: In this study, we re-analyzed the GSE58470 microarray dataset which measured the effect of cisplatin treatment on the expression profile of a cisplatin-sensitive ovarian cancer cell line(IGROV-1) and a cisplatin-resistant cell line (IGROV-1/Pt1). In quality check, the samples were segregated based on their state (sensitive or resistant), indicating the satisfactory quality of this dataset. Comparison of cisplatin-resistant with cisplatin-sensitive samples demonstrated 1931 genes with adjusted P-value < 0.05. ontology (GO) enrichment analysis of DEGs underscored cell cycle, hypoxia pathway, Hippo signaling and 14-3-3 which their role on polyploidy state of cancer cells is determined. On the other hand, analysis of the protein-protein interaction network of DEGs demonstrated key elements of cell cycle regulation like PLK1 as top genes with most degree centrality.
Conclusion: In this study, we followed a systematic approach to exploring the underlying molecular mechanisms of cisplatin resistance in ovarian cancer. PLK1 as a key regulator of the cell cycle was determined as a hub node in our analysis and in accordance with previous studies its down-regulation would be correlated with a cell cycle arrest in G2/M and polyploidy induction. Future studies should be performed to determine the role of PLK1 in ovarian cancer relapse and polyploidy
Keywords: Bioinformatics, Cisplatin, Microarray, Ovarian Cancer, Polyploidy

Ps-2: Microglia Populations in The Brain Cortex, Hippocampus, and Thalamus: A Preliminary Study To Show the Relationship between Their Populations and Neuroregeneration Activity

Afshar AR[*], Farrar Z, Azmoodeh M, Zare N, Mirzaei Sh, Tamadon A

The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Research Institute, Bushehr, Iran
Email: amintamaddon@yahoo.com

Background: Microglia release a range of regeneration factors in the injured nervous system. They are main cells of the immune system in central nervous system and play an essential role in nervous systems development and repair. Microglia relevant to most central nervous system diseases such as migraine, Alzheimer's disease, and Parkinson's disease, are related to cortex, hippocampus, and thalamus, respectively. The aim of our preliminary study was to evaluate the microglia's populations and their microstructures in the cortex, hippocampus, and thalamus of normal mice.
Materials and Methods: Mice brain coronal slices were stained with ionizing calcium-binding adaptor molecule 1 (Iba1) antibody and imaged with confocal microscopy. Different parts of cortex, hippocampus, and thalamus of mice brain slice (five micrometer) were cropped and two dimensionals imaged analyzed using Fiji and Imaris software programs. Various indices such as number of microglia in the 500 μm^2 area, levels of cell branches, and length of cell branches were compared between those three areas of brain. Data were analyzed using SPSS 22 by one-way ANOVA and LSD post hoc tests. $P<0.05$ was considered significant. Data are presented as mean ± SD.
Results: The number of microglia in the 500 μm^2 of cortex (9.3 ± 0.5) was more than the ones in thalamus (6.3 ± 1.5, P=0.01). While the number of microglia in the 500 μm^2 of hippocampus (8.3 ± 1.2) was not different with ones in cortex and thalamus ($P>0.05$). Levels and length of microglia branches were not different between cortex, hippocampus, and thalamus.
Conclusion: Microglia distribution and population differences between cortex, hippocampus, and thalamus may show their importance in occurrence of neurodegenerative diseases in various area of brain. Our study did not show any difference between microstructures of branches of microglia between the cortex, hippocampus, and thalamus.
Keywords: Microglia, Hippocampus, Cortex, Thalamus, Neurodegeneration

Ps-3: Novel Circulating MicroRNA Signature as A Potential Diagnostic Biomarker for Detecting Breast Cancer in The Early Stage

Ahmadi M[1, 2*], Sadeghi H[2], Kamal A[3], Shayesteh Pour B[2], Keller A[3], Hadad P[4], Lashkari M[4], Sharif Zarchi A[5], Kamkar L[2, 6], Totonchi M[2, 7]

1. Department of Genetics, University of Science and Culture, ACE-

CR, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Clinical Bioinformatics, Saarland University, Saarbr¨ ucken, Germany
4. Department of Radiation Oncology, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Bioinformatics, Sharif University of Technology, Tehran, Iran
6. Department of Bioinformatics, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
7. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: m.totonchi@royaninstitute.org

Background: The routine screening for breast cancer (BC) depends primarily on imaging techniques such as mammography, although it is not often sensitive enough for early detection and requires complementary approaches. The discovery of microRNAs (miRNA) has opened a new window for tumor diagnosis. miRNAs are a class of small noncoding RNAs that control gene expression by targeting mRNAs. The aberrant expression is involved in human disease, including breast cancer. The aim of this study was to identify novel biomarkers of circulating miRNAs for detecting BC in early stages.
Materials and Methods: We analyzed the data of miRNAs expression related to 778 patients diagnosed with breast cancer and, 87 control samples, and 5239 healthy and unhealthy samples regarding 13 different types of cancer from The Cancer Genome Atlas (TCGA) database. Twenty-two candidate miRNAs were identified that could distinguish individuals with breast cancer from normal ones based on two bioinformatics approaches: Machin learning and deferentially expression. We used quantitative real-time polymerase chain reaction (qRT-PCR) to validate miRNAs expression levels, in whole blood samples from 71 breast cancer patients and 61 healthy controls.
Results: In this study, we demonstrated that three miRNAs have different expressions in the whole blood of breast cancer patients and non-cancer controls. We used a combination of expressions level of 15 microRNA and 11 life style attributes from demographic data of breast cancer patients and control group. This combination was found to be able to detect breast cancer with AUC: 0.831 in the ROC curve analysis.
Conclusion: These findings suggest that assessment of miRNAs in blood samples can be applied as minimally non-invasive markers for early detection of breast cancer.
Keywords: Breast Cancer, Early Detection, MicroRNA, Biomarker

Ps-4: Computational Analysis of Exerting Oscillatory Fluid Flow into 3D Bone Scaffolds with Different Pore Architectures

Ahmadian B*, Vahidi B

Division of Biomedical Engineering, Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
Email: bahman.vahidi@ut.ac.ir

Background: Stem cells are subjected to mechanical and chemical stimulations to differentiate into the target cell. One of the most common types of mechanical stimulations is the use of bioreactors to induce shear stress on the cell surface. The factors affecting on the shear stress include: scaffold pore and the type of flow applied to the cell. Experiments showed that the use of oscillatory bioreactor facilitates the osteogenic differentiation of stem cells. In this study, distribution of shear stress in 3D bone scaffolds with different pore shapes as a result of oscillating fluid flow has been investigated.
Materials and Methods: Three types of scaffold with spherical, cubical and regular hexagonal pore architectures are modeled in the COMSOL (V 5.3a) software. In these scaffolds, the cube length, radius of the sphere and the hexagonal side length are all assumed equal to 200µm. The fluid under oscillation was assumed as a Newtonian fluid. Inlet fluid velocity was applied as $v= 30 + 300\pi \sin (2\pi t)$ µm/s. outlet boundary condition was assumed as zero-pressure. Computational fluid dynamics was used to solve the problem for the aim of evaluating, distribution of shear stress in 3D scaffolds.
Results: The results show that 87% of the scaffold volume with spherical pores, 82% of the scaffold volume with cubic pores and 97% of the scaffold volume with hexagonal pores are under shear stress ranging from 0.1 to10 mPa. Experimental studies have shown that under this range of shear stress, the stem cell differentiate into bone cell. In addition, the results show that shear stress in the center of the scaffold is negligible and reaches it maximum values near the walls.
Conclusion: It can be concluded from the results that the use of scaffolds with hexagonal pores is suitable for bone tissue engineering applications. Moreover, the central areas of the scaffolds experienced low shear stress (<0.1mPa). So, it's predicted that this area is unsuitable for stem cell placement in order to differentiate into bone cells.
Keywords: Bone Scaffold Geometry, Computational Modeling, Mechanical Modulation, Osteogenic Differentiation

Ps-5: Chick Embryo Provides An Applicable System for Evaluating The Chimera-Forming Ability of Human Pluripotent Stem Cells

Akhlaghpour A[1, 2*], Ghadami SA[2], Molamohammadi S[2], Taei A[2], Kiani T[2], Bahadori Z[2], Samadian A[2], Yakhkeshi S[2], Baharvand H[1, 2], Hassani SN[2]

1. Department of Developmental 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
Email: hossein.baharvand@gmail.com

Background: Human pluripotent stem cells (hPSCs) are defined by their ability to differentiate into derivatives of three embryonic germ layers and displayed two distinct states of pluripotency; naïve and primed. Naïve hPSCs resemble to preimplantation epiblast cells and stand for a higher developmental potential while primed hPSCs correspond to post-implantation epiblast and primed to differentiation. The chimera formation is considered as one of the most stringent assay for evaluation of pluripotency in naïve and primed hPSCs; however, this strategy is challengeable for human cells due to the ethical issues. As an alternative, interspecies chimera formation between hPSCs and an appropriate animal embryo can be a solution. Due to the developmental similarities in the gastrula-stage of chick and human embryos, we assumed that the chick embryo might be a suitable host for receiving hPSCs in interspecies chimera

formation.
Materials and Methods: In this study, we compared the efficiency of interspecies chimerism for naïve and primed hPSCs after injection into the chick embryos at the blastoderm and primitive streak stages, respectively, in the developmental stage matching and non-stage matching approaches. After eight days of injection, the presence of hPSCs's derivatives were traced by GFP detection and immune staining for HNA and three germ layer specific markers also further verified with a sensitive genomic Alu PCR assay.
Results: In the matched developmental stage approach we showed the high ability of both naïve and primed states in participating and differentiating in the developing host embryos. More importantly, we showed that, chick embryos could provide an opportunity for evaluating the hPSCs's pluripotency when they injected into the host embryos as non-developmental stage match approach by survival, proliferation and participation in the developing chick embryos.
Conclusion: Chick embryo can be a suitable host for evaluating the hPSCs pluripotency states and provide provocative approach for *in vivo* differentiation as well as human organoid formation during blastoderm complementation
Keywords: Human Pluripotent Stem Cells (Hpscs), Naive and Primed Pluripotent States, Chick Embryo, Gastrula-Stage, Interspecies Chimera

Ps-6: Hsa-Mir-625-5p Upregulation Promotes Apoptosis in AML Cell Line by Targeting ILK Pathway

Aliabedi B[1*], Mousavi SH[1], Ebrahimi M[2, 3], Alizadeh Sh[1], Hedayati Asl AA[2, 3], Mohammad M[3], Samieyan Dehkordi S[1]

1. Department of Hematology, Faculty of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology & Technology, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology & Technology, ACECR, Tehran, Iran
Email: hmousavi@tums.ac.ir

Background: Growing evidence has demonstrated that microRNAs (miRNAs) have a major effect on development of different types of cancer including AML (Acute Myeloid Leukemia). Expression of miR-625 has decreased in acute myeloid leukemia cell lines.Therefore it seemed that overexpression of miR-625 could decrease tumorigenesis of AML cell lines through ILK (Integrin Linked Kinase) signaling pathway and reducing the associated oncogenes. The aim of this study is to evaluate the effect of hsa-miR-625-5p upregulation on apoptosis and proliferation of KG-1 cell line via ILK signaling pathway.
Materials and Methods: The KG-1 cell line was transfected with pLenti-III-pre mir625-GFP through viral method. Then, overexpression of miR-625-5p as well as the expression level of ILK, AKT, GSK3, c-fos, NF-κB, Caspase 3, Cyclin D1 and stemness genes including KLF-4, OCT-4 and Nanog were analyzed by quantitative PCR (qPCR). Western blotting was used to evaluate of NF-κB, Caspase 3 and p-β-catenin at the protein level. Apoptosis was investigated by Annexin V and flow cytometry. Cell cycle analysis with PI and CCK-8 assay were performed to evaluate proliferation.
Results: Flow cytometric analysis results of KG-1 cells transfected with pLenti-III-pre mir625-GFP construct showed a significant increase in cell apoptosis but no significant alteration in cell cycle. The CCK-8 assay demonstrated that hsa-miR-625-5p doesn't affect proliferation. Gene expression of ILK and NF-κB were downregulated and AKT, c-fos, Caspase3, Cyclin D1, KLF-4, OCT-4 and Nanog were upregulated but no alteration in GSK3 expression profile was observed. Downregulation of NF-κB and upregulation of Caspase 3 and p-β-catenin protein levels were indicated.
Conclusion: miR-625-5p can induce apoptosis but it has no significant effect on proliferation of KG-1 cells. MiR-625 can be a promising approach to aid in the treatment of AML. However, further studies are required in this respect.
Keywords: miR-625-5p, ILK, Apoptosis, Proliferation, AML

P-7: *In Vivo* study of PCL/Lignin Nanocomposite Conduit for Promoting Sciatic Nerve Regeneration in a Rat Sciatic Nerve across a Gap

Amini Sh

Department of Anatomical Sciences and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
Email: sadra.dental@gmail.com

Background: Injuries of the peripheral nervous system (PNS) lead to permanent functional loss of target tissue and neuropathic pain and reduce life quality. The regeneration of damaged peripheral nerve has become a difficult problem. For peripheral nerve regeneration, nerve autograft has been considered as the "gold standard" for the restoration of nerve regeneration. But autograft is limited by the short-term accessibility of donor nerves, increasing illness, and scar formation at the donor site.
Material and Methods: Following the production of PCL/Lignin conduit by Electrospinning technique and Surgical implantation conduit characterized by, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM), electrophysiological assessment, hematoxylin-eosin (HE), Terry Chromosome and toluidine blue staining, Immunohistochemistry (IHC).
Results: This investigates present that PCL/Lignin conduit can have the good accelerating potential for neural length and promoting regeneration sciatic nerve.
Conclusion: Our results propose that PCL/Lignin serve as a new biodegradable artificial nerve guide for nerve tissue engineering.
Keyword: Peripheral Nerve Injuries, Biodegradable Nerve Conduit, Electrospinning, Sciatic Nerve Regeneration

Ps-8: Establishment of Human Embryonic Kidney-239 Cell Line with High-Stress Resistance and Protein Expression Capacity

Amiri F[1*], Abbasi-Malati Z[3, 2], Habibi Roudkenar M[3]
1. Department of Medical Laboratory Sciences, School of Para medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
3. Medical Biotechnology Department, Paramedicine faculty, Guilan University of Medical Sciences, Rasht, Iran
Email: roudkenar@gums.ac.ir

Background: High yield production of recombinant proteins with proper glycosylation, folding, solubility and bioactivity is one of the most important challenges in the field of bio-pharmaceutical industries. Human embryonic kidney-239 (HEK-

239) cell line is used as mammalian expression system for recombinant protein production. Any manipulation to improve ability of this cell line for expression of desired recombinant proteins is great of interest. NFE2L2 gene, as master gene in cellular redox hemostasis, and its transcription factor, nuclear factor E2-related factor-2 (Nrf-2), play important role in cell proliferation and oxidant/ antioxidant balance. Here in we established Nrf2-overexpressing-stable HEK293 cell line with UV-resistance capacity for future using in term of recombinant protein production.
Materials and Methods: HEK-293 cell line was transfected with Nrf2 containing pcDNA3. geneticin was used to select stable clones. RT-PCR and western blot were done to confirm Nrf2 expression after transfection. Water-soluble tetrazolium salts-1 (WST-) assay was performed to determine proliferation rate of UV irradiation stress induced HEK-239. The expression of Quinone Oxidoreductase 1 (NQO1) and certain glutathione S-transferases (GSTs), as Nrf2 downstream genes, was evaluated by real-time PCR in UV exposed HEK-293 and HEK-293.
Results: Transfected HEK-293 expressed Nrf2 gene at transcriptional and translational levels. WST-1 results indicated that the Nrf2-overexpressing HEK293 showed higher proliferation rate even after exposure to UV irradiation in comparison with non-transfected ones. High expression levels of NQO1 and GSTs were detected in transfected and UV stress induced HEK-293.
Conclusion: This Nrf2-overexpressing-stable HEK293 with higher growth rate augmented for more resistance against stress condition could use for high yield production of recombinant proteins.
Keywords: HEK-293 Cell Line, Nrf2, Recombinant Protein

Ps-9: No Titletherapeutic Properties of Mesenchymal Stem Cell Secretom or Silybum Marianum Extract on Ccl4- Induced Liver Failure Mice

Amiri F*, Molaei S

School of Medicine, Qom university of Medical Sciences, Qom, Iran
Email: amiri2012@gmail.com

Background: Due to some limitation in term of liver disease treatments including restriction to adequate donated liver and complication of liver transplantation, applying of new curative approaches are more valuable. Recently, mesenchymal stem cell (MSCs) and their derivatives including macrovesicle, harvested conditioned medium and exosome are used to treat different sever diseases. MSC conditioned medium contains several cytokines, growth factors and other bio-reactive molecules. On the other hand, Herbal extract is usually in term of disease treatment. In this regard we conducted this study to compare the herbal Silybum marianum extract to MSC-conditioned medium (MSC-CdM) therapeutic potentialities as a potential novel strategy in term of liver failure treatment.
Materials and Methods: Silybum marianum extract was purchased and human amniotic membrane-derived MSC-conditioned medium was harvested and sterilized using 0.22 μm filters. CCl4 induced-liver failure mice models (LF) was established and confirmed by biochemical and histological analysis according to our previous study. 4 animal groups containing 10 mice, were designed as follows, normal mice receiving phosphate buffer solution (PBS) as normal control, LF-receiving PBS as sham, LF- receiving Silybum marianum extract as LF-Sm and LF- receiving MSC-CdM as LF-CdM. All treatments were done 24 hours post CCL4 injection. Biochemical, histopathological and 14-days-survival rate assay was applied to evaluate hepatoprotective properties of Silybum marianum extract and MSC-CdM in different interval times, 2, 4, 7, 10, 14 days after administration of Silybum marianum extract and MSC-CdM.
Results: According to biochemical assay 2 and 4 days post-treatment, alanine aminotransferase (ALT) Aspartate transaminase (AST) were significantly increased in the sham group comparing to the normal group, however, these mentioned enzyme values were significantly lower in the LF- Sm, and LF-CdM groups compared to sham ($P<0.05$). The histopathological assay showed a dramatically decreased number of inflammatory cells and presence of repaired cells in LF- CdM mice. LF-Sm mice revealed mild necrosis and inflammation compared to bridging necrosis in sham. Next, comparing LF- Sm group to LF-CdM group, significantly lower values of AST was detected 4 days after injection in LF-CdM group. However, these two groups showed no significant difference in liver enzyme levels 2 days after injection. LF-CdM showed rapid liver control and less mortality rate compared to other experimental groups.
Conclusion: Both Silybum marianum extract and MSC-conditioned medium have demonstrated hepatoprotective properties, however less mortality rate in LF-CdM showing a promising future an adjuvant therapy for liver disease.
Keywords: Mesenchymal Stem Cell, Conditioned Medium, Silybum Marianum, Liver Failure

Ps-10: Nrf2 Pathway-Related Micrornas in Mesenchymal Stem Cell: New Approach in Regenerative Medicine

Amiri F[1, 2*], Molaei S[2]

1. Department of Medical Laboratory Sciences, School of Para Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2. Qom University of Medical Sciences and Health Service, Qom, Iran
Email: f.amiri@umsha.ac.ir

Background: In recent decades, with introducing of stem cells including mesenchymal stem cells (MSCs), huge body of medical publications have focused on different applications of these mentioned useful cells in the field of regenerative medicine. However some limitations such as MSCs high death rate in vivo/ *in vitro* and susceptibility to oxidative stress are some of existing problems in the field of MSC-based cell therapy. Hence, application of practical and simple approaches to address these challenges are necessary. One of this strategy is manipulation of MSCs with cytoprotective gene, nuclear factor E2-related factor-2 (Nrf2). This study was aimed to evaluate Nrf2-related MicroRNAs expression in MSCs that might involve in oxidative stress response in these mentioned valuable cells.
Materials and Methods: Umbilical cord MSCs were cultured in specific culture media and transfected with recombinant plasmid construct containing Nrf2 using Fugene HP reagent. The expression level of some related MicroRNAs including miRNA 29a, miRNA 206, miRNA 34a and miRNA 153 was assayed using RT-PCR and Real time PCR in transfected MSCs. Non-transfected MSCs was considered as control.
Results: RT-PCR and western blot confirmed Nrf2 expression in the transfected cells. Expression of miRNA 29a was up regulated in Nrf2-overexpressed MSCs compared with the control group. miRNA 206 was not expresseddi-erentially in both subjected groups. Transfected MSCs with ability to produce Nrf2

transcription and translation biomolecule showed decreased expression levels of miRNA 34a and miRNA 153 in comparison with control.
Conclusion: Manipulation of MSCs with Nrf2 down regulates the expression of some miRNAs, miRNA 34a and miRNA 153, that suppress its expression at translational level. This alteration in miRNAs expression might result in development of MSCs antioxidant capacities and prime them for harsh stress condition.
Keywords: Mesenchymal Stem Cells, Nrf2, Oxidative Stress, MicroRNAs

Ps-11: Assessment Of Toll Like Receptor 4 Activation in HEK Transformed Cell Line via Incubation with Semen from Asthenozoospermia Patients

Asfa S[1*], Ghaedi K[2], Peymani M[2], Nasr-Esfahani MH[2]

1. Department of Biology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
2. Department of Cellular Biotechnology, Cell Science Research, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: kamranghaedi@royaninstitute.org

Background: Infertility could disrupt the essential steps of human life as an important disorder in the evolution pathway and results in an extensive trauma. Asthenozoospermia is one of the prevalent causes of male infertility which could be a result of infection. Infection reduces the sperm motility and causes the male infertility. In the studies carried out on semen and sperm, it is clarified that bacterial endotoxins such as the LPS of gram-negative bacteria, activates the TLR4 pathway and induces the expression of Chemokines and Cytokines coding genes. Consequently, it has an important role in apoptosis induction and sperm motility reduction. Sperm motility reduction often occurs as a result of infection and TLR4 induction. According to this fact, the goal of this study is to benefit from a suitable cellular model such as transfected human embryonic kidney (HEK) cells with TLR4, in order to study the effects of asthenozoospermia patients' semen and to prove the existence of specified infection for this receptor from assessment of gene expression TLR4 downstream of iNOS and TNF-α in asthenozoospermia patients.
Materials and Methods: Semen samples of 15 in asthenozoospermia patients and 15 healthy person who refer to the Isfahan fertility and infertility clinic, were examined. Motility of samples was studied according to the WHO 2010 protocol. LPS was studied as the positive control. Transfected HEK cells with TLR4 were treated with different concentrations of semen. After 24 hours, in order to assess the afformentioned genes, mRNAs were extracted by means of TRIZOL, cDNAs were synthesized and then Real time PCR was carried out. Results: Significant increment in expression of downstream genes of TLR4 receptor including iNOS and TNF-α in asthenozoospermia patients in comparison to healthy people was found out.
Results: Significant increment in expression of downstream genes of TLR4 receptor including iNOS and TNF-α in asthenozoospermia patients in comparison to healthy people was found out.
Conclusion: In conclusion, infection induces the activation of the TLR4 pathway, reduces the sperm motility and results in infertility
Keywords: Asthenozoospermia, Transfected HEK Cells, TLR4 Pathway, Infection, LPS

Ps-12: Mesenchymal Stem Cells Derived from Embryonic Stem Cell -Conditioned Medium Facilitates Neuroprotection through Enhancement of Neurogenesis on Ischemic Stroke Model

Asgari Taei A[1*], Farahmandfar M[1], Hassanzadeh GhR[2], Dargahi L[3], Nasoohi S[3], Kadivar M[4]

1. Department of Neuroscience and Addiction Studies, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Neurobiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Biochemistry Pasteur Institute of Iran, Pasteur Institute of Iran, Tehran, Iran
Email: asgari.afsane@yahoo.com

Background: Mesenchymal stem cells have emerged as a potential therapy for a range of neural insults. Although low survival rates and potential tumorigenicity of implanted cells could reduce the efficacy of cell-based treatment, recent investigations have proven that use of stem cell conditioned medium (CM) may be a feasible approach to overcome these limitations. There are numerous cytokines and growth factors in the CM of various stem cells responsible for paracrine protective effects of stem cells. The neurogenesis is crucial for the recovery of neural functions after stroke. The purpose of the present study was to assess the effect of conditioned medium of mesenchymal stem cells on neurological functions, infarct volume and neurogenesis in ischemic stroke rats.
Materials and Methods: Ischemic stroke was induced by standard right middle cerebral artery occlusion method (MCAO) in 8-week old Wistar male rats. Injection of the CM or DMEM (5µl) was respectively done into the left lateral ventricle of treatment and control animals from one hour following the surgery to the second day after MCAO induction (three doses). Behavioral tests were performed on 1, 3 and 7 days after injury. Infarct volumes were evaluated on 3 and 7 days after the MCAO. The expression of Nestin, Ki-67 and Doublecortin mRNAs and proteins in the subventricular zone (SVZ) and subgranular zone (SGZ) regions was assessed using Real-time PCR and immunolabeling at 7 days after MCAO.
Results: Our results indicated that 90 minutes occlusion of the artery caused impairments in sensory-motor functions in ischemic rats. Furthermore, i.c.v injection of the CM improved functional recovery in treatment group after stroke. Volume analysis revealed that animals subjected to treatment with CM had significantly smaller infarcts than controls. Treatment with CM significantly increased number of Nestin, Ki-67 and Doublecortin-positive cells in ipsilateral hemisphere on the 7th day.
Conclusion: Our data suggest that conditioned medium of mesenchymal stem cells contributes to neuroprotection following a cerebral ischemia insult through an improvement in neurogenesis.
Keywords: Conditioned Medium, Ischemic Stroke, Neurological Function, Infarct Volume, Neurogenesis

Ps-13: Electroluminescence Cell Imaging Using A Novel Synthesized Cadmium Sulfide Quantum Dot

Askari N[1*], Asakari MB[2]

1. Research Department of Biotechnology, Institute of Sciences

and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran
2. Department of Physics, Faculty of Science, University of Guilan, Rasht, Iran
Email: nahid.askari@yahoo.com

Background: Cadmium sulfide is one of the most widely used materials, due to its widespread use in optical instruments, solar cells, optical sensors, and bio-sensors.
Materials and Methods: In this study, Cadmium Sulfide Quantum Dots (CdSQD) were successfully synthesized using an easy hydrothermal method with an average size of 6 nm. The synthesized CdSQD were characterized by Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), Energy-dispersive X-ray spectroscopy (EDX), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). The optical properties of the CdSQD nanocomposites were measured in addition to its applications in cell imaging and photoluminescence. Fluorescent synthesized CdS in aqueous solutions of MEMα medium has been optimized (1µg/ml) and The fluorescent properties of CdS used to monitor its uptake in HEK-293 cells. In this regard, cells were incubated with 1 µg/mL CdS for 3 h and then washed to remove any unbound Cds. It was observed the fluorescence pattern in most of HEK-293 cells when examined under fluorescence microscopy.
Results: It has been demonstrated that such CdS nanoparticles can be used to image cell cultures and study the cell structure
Conclusion: Synthesized quantum dot particles due to their small size, have high cell penetration, and this property can be used for pharmaceutical studies in medical science that avoids the side effects of some drugs, such as anticancer drugs. Because these drugs should not be used at high doses to reduce damages so to the healthy cells of the body.
Keywords: Cadmium Sulfide Quantum Dot, Hydrothermal Synthesis, Photoluminescence, Electroluminescence, HEK-293 Cells

Ps-14: Improvement of Optic Vesicle Formation during Retinal Organoid Development Using L-Ascorbic Acid

Avoj S[1, 2*], Karamali F[2], Karimi A[1], Nasr-Esfahani MH[2]

1. Department of Biology, University of Payam Noor, Tehran, Iran
2. Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mh.nasr-esfahani@royaninstitute.org

Background: The specific properties of pluripotent stem cells (PSCs) provide a suitable tool to study developmental events *in vitro*. Recent advances in 3D technology help to prepare a new aggregate formation method called "organoid" which mimics in vivo developmental events. Retinal organoid is one of the pioneers organoid field. Recently numerous studies have offered to improve the first method of retinal organoid.
Materials and Methods: In this study, by introducing the propose factors into the first step of retinal organoid formation we modified the previous culture medium according to probable problems initiated by dissociation of human embryonic stem cells (hESCs).
Results: According to our results, using L-ascorbic acid as an antioxidant agent and reducing knockout serum replacement (KSR) concentration, could increase the integrity of aggregates in comparison to the control group. We started a second medium containing fetal bovine serum (FBS), retinoic acid and N2 for one more week. Our data correspond to improve retinal organoid formation. The cells were organized into cyst-like structures with a columnar epithelium-lined lumen and also developed the optic vesicle structure.
Conclusion: Although a number of recent studies believe that despite optic vesicle some parts of body organoids express specific markers of retinal differentiation, but the optic vesicle formation is still considered as a critical phenomenon for retinal development *in vitro*. Our results revealed that presence of antioxidant component can recover the individual hESC condition to further following stages and final results, and will facilitate an improved method for future developmental studies.
Keywords: L-Ascorbic acid, Optic Vesicle, Retinal Organoid

Ps-15: Cardiac ECM Hydrogel Coating with A Compound Cocktail Induce Hadscs to Cardiomyocyte Like Cells

Baghalishahi M[1*], Eftekhar-Vaghefi SH[2], Piryaei A[2], Nematollahi-Mahani SN[2], Mollaei HR[2], Sadeghi Y[2]

1. Department of Anatomical Science, School of Medicine, Afzalipour University of Medical Science, Kerman, Iran
2. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: fbaghalishahi@gmail.com

Background: The researches demonstrated that inductive factors alone were not sufficient for the differentiation of stem cells into cardiomyocytes. Recently, cardiac extracellular matrix (cECM) coating provided tissue specific microenvironment and act as inductive template for efficient cell differentiation. The aim of this study was to investigate the effect of cardiac ECM (cECM) on cardiomyocyte-like cell differentiation of human adipose tissue-derived stem cells (hADSCs) using cECM hydrogel in combination with a cardiac inductive cocktail.
Materials and Methods: After approval of characteristics of hADSCs and cECM hydrogel, hADSCs culture on coated ECM with and without inductive cocktail for 3weeks. qRT-PCR and western blot were used for evaluating the expression pattern of special cardiac genes and proteins.
Results: Our study showed enhanced expression of GATA4, HAND1, HAND2, NKX2.5, Troponin I, βMHC, Connexin43 genes, and Connexin43, cTnI and βMHC proteins.
Conclusion: We confirmed that cECM by itself could affect viability, proliferation and differentiation of hADSCs. However, using cECM in combination with a cardiac inducing cocktail improved the results. Also hADSCs can be considered as an appropriate source for cardiac tissue engineering.
Keywords: Cardiac Extracellular Matrix, Tissue Engineering, Cardiomyocyte-Like Cell, Human Adipose Tissue-Derived Stem Cells, Differentiation

Ps-16: Cis p-Tau Mediates Neurodegeneration Upon Hypothermia

Bagheri kakolaki F[*], Shahpasand K

Department of Brain and Cognitive Sciences, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: shahpasand@royaninstitute.org

Background: Alzheimer's disease is the sixth leading cause of death globally and is a multifactorial disorder. Misprocessed

amyloid precursor protein and hyper-phosphorylated tau are the major pathological hallmarks playing part in AD. It is clear that hypothermia results in AD the mechanism of which has remained unsecure thus far. It has been recently reported that phosphorylated tau at Thr231 exists in the two distinct cis and trans conformations; whose conversion is being mediated by Pin1 isomerase. Pin1 converts cis to trans species and its suppression reflects cis p-tau accumulation and neurodegeneration. We herein examined if hypothermia may suppress Pin1 function.
Materials and Methods: In this study, we examined hypothermia effects on cis p-tau accumulation in SH-SY5Y cells using immunoflourescent (IF) and western blotting (WB) techniques. We treated 5 cell groups under 5 different temperature condition 39,37,30,20 and 15 degrees Celsius in tree times 24,48,72 hours. Also,the expression level of Pin1 enzyme was assessed using real time polymerase change reaction (RT-PCR).
Results: We found hypothermia induces cis p-tau accumulation and neurodegeneration in cultured cells.Morever, we determined that Pin1 is being inhibited upon hypothermia wherby reflecting cis p-tau accumulation and neurodegeneration.
Conclusion: Our findings unravel tauopathy mysteries upon hypothermia and would help us find and efficient therappan of patients; likely through immunotherapy with cis pT231-tau monoclonal antibody.
Keywords: Alzheimer's Disease, Hypothermia, Tauopathy,Cis P-Tau

Ps-17: Endogenous Wnt/ β catenin Signaling Pathway Is Inhibited During Osteogenic Differentiation of Adipose-Derived Mesenchymal Stem Cells

Baghernezhad Shayan F[1*], Parvaneh Tafreshi A[2], Zeynali B[1]

1. Labaratory of Developmental Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Medical Biotechnology, Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: zeynalib@ut.ac.ir

Background: Bone repair is one of the most important and urgent needs for the society to overcome the problems concerning the skeletal diseases. Nowadays, cell therapy is one of the hopeful methods. Studies have shown that adipose-derived mesenchymal stem cells (ADMSCs) can be induced towards the osteogenic differentiation both *in vitro* and *in vivo*. Among different signaling pathways involved during this process, Wnt/β-catenin plays a significant role at several stages of osteogenesis. There are contradictions however, some of which reveal the osteo-induction but others point at the osteo-inhibition. Our previous results favored the inhibitory effect of the exogenously activated Wnt pathway during osteogenesis. This prompted us to examine how the endogenous Wnt/β-catenin pathway is being altered during osteogenesis of ADMSCs.
Materials and Methods: ADSCs were isolated from a 36 year old woman and cultured in high glucose DMEM supplemented with 10% FBS, until 80% confluency. They were then treated with dexamethasone, ascorbic acid and β-glycerol phosphate as the osteogenic differentiation medium. Alizarin red staining and calcium content were evaluated on days 7, 14 and 21 post treatment. To analyse the activity of endogenous Wnt/β-catenin signaling pathway during the osteogenic differentiation, real time PCR was performed for: 1) the Wnt related genes such as cyclin D1 and Wnt3a; 2) DKK1, as the Wnt antagonist; and 3) the osteopontin as the latent osteogenic gene.
Results: Our results showed that the amounts of extracellular matrix calcification and calcium deposition were gradually increased during the 21 days of treatment with the osteogenic medium. Coincidentally, the expressions of DKK1 and the osteopontin were up regulated whereas those of the Wnt3a and cyclin D1 decreased significantly.
Conclusion: Our results suggest that ADSCs has the potential to differentiate into the osteogenic lineage during which the endogenous Wnt/β-catenin pathway is inhibited especially at the later stages of the osteogenesis.
Keywords: Wnt/β-catenin, Adipose Derived Stem Cell, Osteogenic Differentiation

Ps-18: Planarian Promoter Architecture

Bagherzadeh R[1, 2, 3, 4, 5, 6*], Minkina E[6], Deviatiiarov R[6], Sharifi-Zarchi A[1, 5], Gusev O[6, 7], Baharvand H[1, 4], Agata K[2, 3]

1. Department of Biophysics, Kyoto University, Kyoto, Japan
2. Department of Life Science, Gakushuin University, Tokyo, Japan
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 Computer Engineering, Sharif University of Technology, Tehran, Iran
5. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
6. Extreme Biology Laboratory, Institute of Fundamental Medicine and Biology, Russia
7. Division of Genomic Technologies, RIKEN Center for Life Science Technologies (CLST), Yokohama, Japan
Email: RezaBagherzadeh.email@gmail.com

Background: Core promoters are minimal regions sufficient to direct accurate initiation of transcription and are crucial for regulation of gene expression. They are highly diverse in terms of associated core promoter motifs, underlying sequence composition and patterns of transcription initiation. Distinctive features of promoters are also seen at the chromatin level, including nucleosome positioning patterns and the presence of specific histone modifications. Freshwater planarian, Dugesia japonica and Schmidtea mediterranea have emerged as powerful model system for studying regeneration and adult stem cell (ASC) transcriptional and chromatin biology research. Such research is contingent on the accurate annotation of transcription start sites (TSSs) and promoters.
Materials and Methods: To characterize the cis-regulatory landscape of D. japonica as the first record of TSSs activity genome-wide, we produced a high-resolution map of the promoters active in D. japonica and S. mediterranea. We performed Cap Analysis of Gene Expression (CAGE) and the Identified TSSs were utilized to generate a first record of "Planarian Promoter Database," as a catalog of active promoters across the surveyed samples.
Results: We characterized and compared the features of promoters and identified candidate core promoter motifs for FACS sorted populations and integrated de novo motif discovery using CAGEdefined TSSs. A comparison of promoter activities, motif discovery and differential gene expression revealed insights into the properties of stem cell regulation and differentiation.
Conclusion: Our planarian Promoter Database presented here provides a valuable resource for the comparative study of cis-regulatory regions, as well as a fundamental resource data for

transcriptional, gene regulatory and epigenetic studies.
Keywords: Transcription Start Site, Promoter, CAGE-Seq, Stem Cell and Regenerative Biology, Planaria

Ps-19: Efficient Differentiation of Human Pluripotent Stem Cells into Dendritic Cells by Chemical Approach

Barati M[1, 2*], Sabahi Moosavi N[1, 2], Janzamin E[2], Rassouli H[2], Ghodsi A[2], Baharvand H[2], Ebrahimi M[2], Hassani SN[2]

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

Background: Since patient condition affects the function of dendritic cells (DCs), developing fully functional DCs from human pluripotent stem cells (hPSCs) has been promising for DC-based immunotherapy. Here we established a new protocol for efficient production of hPSCs-derived DCs.
Materials and Methods: Using 3D culture condition and small molecules, hPSCs were committed and differentiated into haematopoietic progenitor cells (HPCs). Then, HPCs were induced to generate DCs and analyzed for morphological and functional properties.
Results: In order to efficiently derive DCs from hPSCs, we first generated HPCs. To this, hPSCs were induced to differentiate as aggregates in the presence of CHIR99021 and ascorbic acid small molecules. In this protocol, we observed that most of the aggregates tend to cavitate and form cystic body. Second, the cystic bodies were plated onto a matrigel-coated dish and cultured in expansion media in order to release non-adherent haematopoietic-like cells. The released cells possessed robust myeloid colony forming activity. Third, we collected non-adherent cells and transferred them into the ultra-low attachment plates containing DC differentiation media and observed efficient DC-like cells formation. Flowcytometry assay, scanning electron microscopy, Wright-Giemsa staining demonstrated that hPSC-derived DCs produced by this protocol were comparable with human peripheral blood monocyte-derived DCs in terms of cell surface receptors and morphology. In total, we were able to generate 1.5×10^6 DCs from 105 PSCs. Furthermore, independent hPSC lines were used to show reproducibility of the protocol.
Conclusion: We assumed that 3D culture condition more closely imitates natural tissue development than 2D culture systems. To corroborate this hypothesis, we established a protocol in 3D culture condition with the presence of small molecules. Our results suggest that this approach can be effective in production of DCs and can be useful in many fields including human cancer immunotherapy.
Keywords: Pluripotent Stem Cell, Haematopoietic Stem Cell, Small Molecules, Dendritic Cells

Ps-20: Brittle Star's Arm Regeneration: A Preliminary Gas Chromatography–Mass Spectrometry Study of Molecules Playing Roles in whole Tissue Reconstruction

Basouli N[*], Keshtkar MR, Mohebbi GhH, Barmak AR, Khataminejad M, Bargahi A, Azari H, Nabipour I, Tamadon A, Mahmoudpour M

The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran
Email: nahidbasouli@gmail.com

Background: Brittle stars is one of the marine invertebrates has the ability of whole arm reconstruction after arm amputation. However, based on our knowledge there is no information regarding bioactive chemical molecules have roles in brittle stars' tissue regeneration. The aim of the present study was to identify molecules have roles in tissue regeneration in brittle star using gas chromatography–mass spectrometry (GC/MS) during the first week of amputation.
Materials and Methods: Specimens of adult brittle stars ranging from 15-20 cm in overall diameter, were collected and maintained in aquaria containing aerated saltwater at 25°C with a timer-controlled light cycle with 14 hour light and 10 hours dark. Brittle stars were randomly allocated into four groups: intact group and 3 hours, 3 days, and 7 days after arm cut groups. In the arm amputated groups, two third of one arm-tip was cut from each brittle star and they were kept in aquarium three hours, three days, and seven days before chopping. Forty grams of whole body of intact and arm amputated brittle stars were chopped and extracted by 200 ml of 70% ethanol solution for 5 days. The hydro-alcoholic extracts were analyzed by GC/MS.
Results: Twenty-four compounds detected in hydro-alcoholic extract of intact brittle stars. Three hours after cutting arm, three new compounds were appeared in the analysis compared to the compounds of intact brittle stars. They were suspected to have antiseptic properties such as colchicine, N-desacetyl-N-[4-acetoxy-3,5-dimethoxycinnamoyl]. Furthermore, three and seven days after cutting the arms, the hydro-alcoholic extracts had 11 different compounds compared to the extract of the three hours after cutting the arms. Four compounds were fatty acids such as linoleic acid ethyl ester, docosahexaenoic acid, 1,2,3propanetriyl ester, and 1-dodecanol,3,7,11-trimetyl, cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5alpha) have anti-proliferation properties and can induce apoptosis. The other detected compounds had unknown biologic effects.
Conclusion: GC/MS analysis of hydro-alcoholic extract of brittle stars during the first week of arm regeneration showed that brittle star produces antiseptic and pro-apoptotic molecules which may help it to remove microbial infections and remove excess cells in the healing arm to make a basement for new arm regeneration.
Keywords: Regeneration, Gas Chromatography–Mass Spectrometry, Hydro-Alcoholic Extract, Brittle Star,

Ps-21: Marine-Based Scaffold 3D Bio-Printing System Design: A Preliminary Study

Behrouzi P[1*], Muhaddesi M[1], Hashemi AR[1], Khamisipour GhR[2], Javadzade H[3], Almasi-turk S[4], Khoshkhoo A[5], Tamadon A[1]

1. The Persian Gulf Marine Biotechnology Research Center, Bushehr University of Medical Sciences, Bushehr, Iran
2. Department of Hematology, Faculty of Allied Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
3. Department of Health Education and Health Promotion, Bushehr University of Medical Sciences, Bushehr, Iran
4. Department of Anatomy, Faculty of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
5. Department of System Science and Industrial Engineering, Bing-

hamton University, Binghamton, USA
Email: amintamaddon@yahoo.com

Background: Bio-printing has been emerged as a developing technology for human tissues reconstruction. Tissue engineering and organ printing extremely depend on the development of an implicated 3D printing system which has the ability to accurately control the parameters. Bio-ink is the other most important part of a 3D printed scaffold. The purpose of this preliminary study was a 3D bio-printer development and printing a bone scaffold using a marine-based bio-ink.
Materials and Methods: Extruded deposition modeling (EDM) is utilized to setup the electromechanical part of the 3D printer possessing three-axis movement in X, Y and Z directions with micro-scale high resolution motion. A hydrogel marine-based bio-composite which encompassing living cells is exploited as a bio-ink to 3D print a bone scaffold. Accordingly, the extrusion process needs to be carried out through a mounted extruder on X axis with a highly accurate hydrogel extrusion. Needle size, syringe diameter and nozzle temperature were the parameters that effect the printing process and form the fundamentals of extrusion kit design for bio-printer.
Results: The designed extrusion kit contains a nozzle that enables the user to 3D print a composite and geometrically complex biological scaffolds. We also developed an alginate-hydroxyapatitebased bio-ink to print the bone scaffold. Various factors such as the movements' accuracy and bioink extrusion were controlled via electronic sensors. The particular parameters were measured and sent to a central processing unit (ATmega2560) to be processed. 3D CAD files were fed to the control unit for slicing and G-code generation to control the axes movement in order to 3D print complex biological scaffolds.
Conclusion: Using our developed 3D printer and the marine-based bio-inks we are now able to 3D print the biological organs and living scaffolds such as bone.
Keywords: 3D Printer, Alginate, Hydroxyapatite, Bio-Ink, Marine

Ps-22: The M2c Macrophages Promote Phalange Regeneration in An Organ Co-Culture with Amputated Mice Digit

Bijarchian F*, Taghiyar L, Baghaban Eslaminejad MR

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

Background: Delayed anti-inflammatory responses and subsequent fibrotic scarring is the main causes for the fail to regrow of injured body parts such as limb/phalange in adult mammalians. Indeed, fully scar-free limb regeneration in adult salamanders, which can restore complete body parts, followed by appearance of anti-inflammatory macrophages in the site of injuries during the first 24 hours, after amputation. Consequently, modulation of inflammatory responses may provide valuable therapeutic approach for mammalians limb regeneration. Hence, this study aimed to analysis the local effects of IL-10 priming M2c macrophages on the digit tip regeneration of fetal mice forelimb in a co-cultured organ system, after amputation.
Materials and Methods: To address this evaluation, we obtained hands of 18.5E-day-old C57BL/6J fetal mice, which amputations performed on digits. The mononuclear cells such as monocytes were obtained from C57BL/6J mice peripheral blood, cultured and enhances the phenotype of M2 macrophages subsequent induced by IL-10. Then, amputated hands co-cultured with M2c macrophage. In addition, some of samples treated with 40, 80 and 120 μg/ml concertation of IL-10 and TGF-β1cytokines as the main M2c macrophages secretions, respectively.
Results: We found that wound closure occurred in all specimen, three days' post amputation (dpa). Increasing of the digit elongation achieved at 10 dpa in all groups that was associated with increased expression of specific markers of regeneration such as Msx1, Msx2 and Bmp4 (**P<0.01). In addition, enhanced proliferation rate observed that is related with high level expression of Fgf-8, Ki67 (**P<0.01). Histological analyses indicated epidermal closure happened 3 dpa in all groups. H&E staining showed that apoptotic cell not identified in all groups. Surprisingly, full digit tip regenerated in co-cultured group. Indeed, Alizarin red-Alcian blue staining revealed new callus formed colored blue and rare bony-like tissue formation observed that colored red by alizarin red. Particularly, new tissue regrowth observed in 40 μg/ml IL-10 and 120 μg/ml TGF-β1 compared with control. In contrast, in control formed no remarkable digit elongation, as expected.
Conclusion: We propose that existence of anti-inflammatory macrophages in injury site of digit can regenerate epithelial and mesenchymal tissues may actually be employed in limb regeneration without scar formation in adult mammalian.
Keywords: TGF-β1, IL-10, Limb Regeneration, M2c Macrophage, Amputation

Ps-23: To Study DAPK1 Effects on Tauopathy in The Stressed out Culture Neurons

Dadzadi M[1*], Shahpasand K[2], Mousavi SE[3]

1. Department of Biotechnology, Faculty of Advanced Science and Technology, School of Medicine, Islamic Azad University, Tehran, Iran
2. Department of Brain and Cognitive Sciences, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Emails: maedeh.dadzadi@gmail.com; shahpasand09@gmail.com

Background: Tau abnormal hyperphosphorylation is a major pathological hallmark in neurodegeneration. It has been shown that phosphorylated tau at Thr231 exists in the two distinct cis & trans conformations, in which that cis p-tau is extremely neurotoxic and drives neurodegeneration. The conversion of cis p-tau to trans is being mediated by Pin1 isomerase. Pin1 can be inhibited by multiple mechanisms, resulting in cis p-tau accumulation and neurodegeneration. On the other hand, DAPK1 (death associated protein kinase 1), has a key role in the development of Alzheimer disease; likely through Pin1 suppression. It has been shown that nutrition depletion stress would result in cis p-tau accumulation in SH-SY5Y cells. We herein examine if DAPK1 inhibition may stop cis p-tau accumulation in the cells.
Materials and Methods: We immunostained stressed out SH-SY5Y cells in both treated and untreated with DAPK1 inhibitor with cis pT231-tau monoclonal antibody. Also, by Western Blotting Technique, we studied the amounts of cis p-tau and Pin1 in the both groups.
Results: We found a cis p-tau increase in stressed out SH-SY5Y cells in a timely manner, leading to the cell death. Importantly,

we found that DAPK1 Inhibition leads to a reduced cis p-tau levels as well as suppressed neural cell death.

Conclusion: Treating stressed out SH-SY5Y cells with DAPK1 inhibitor not only decreases cis p-tau amounts but also prevents neuronal apoptosis. These results open new windows toward neurodegeneration molecular mechanisms in which DAPK1 plays central role. We believe that DAPK1 inhibitors may have therapeutic implications in cis p-tau reduction and AD treatment.

Keywords: Alzheimer's Disease (AD), Tauopathy, Pin1, DAPK1, DAPK1 Inhibitor

Ps-24: The Effects of Microvesicles Released by Embryonic Carcinoma Stem Cells Treated with Retinoic Acid on Neural Differentiation of Mesenchymal Stem Cells

Danaeinezhad Sh[1*], Babashah S[2], Fahimi H[3]

1. Department of Molcular Genetics, Islamic Azad Tehran Medical Science University, Tehran, Iran
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Genetics, Faculty of Advanced Science and Technology, Islamic Azad University Tehran Medical Branch, Tehran, Iran
Email: sh.danaei.n@gmail.com

Background: Neurogenesis function is a limited process, so diseases associated with neurodegenerative diseases are a major problem. Replacing nerve cells instead of destroying cells to treat these diseases is a worthwhile solution. Therefore, the neural differentiation of mesenchymal stem cells can be a reasonable way to achieve this goal. EXOSOMES are nanovesicles of endocytic origin that have important role in extracellular communication and potential usage for tissue regeneration. Exosomes have the potential to carry and deliver MicroRNAs.

Materials and Methods: In this study, firstly embryonic carcinoma cells with retinoic acid and cocktail differentiated into neuronal cells and their differentiation was confirmed by Contrast phase microscopy images. The peripheral environment of embryonic carcinoma cells was collected for the isolation of exosomes and exosomal isolation was performed using differential centrifugation and ExoQuick, exosome precipitation solution protocol. Exosomes were identified by an electron microscope and confirmed by dynamic light scattering (DLS). Mesenchymal stem cells were treated with NT2-derived exosomes. Finally, mesenchymal stem cells changed morphology and then followed the process of apparent bipolar and multipole differentiation. Immunocytochemistry and real time PCR techniques were used to evaluate the expression of neuronal markers.

Results and Conclusion: This study was the first evidence of the finding that exosomal microvesicle cells, secreted from neuronal differentiation cells, can induce neural differentiation in mesenchymal stem cells.

Keywords: Microvesicles, Exosomes, Stem Cells, Embryonic Carcinoma Cells, Neural Differentiation

Ps-25: Cytotoxicity Effect of Trans Cinnamic Acid on Hela Cancer Cell Line

Darya Gh[1*], Soleimani L[2], Moayedi Rad M[3], Heydari H[4], Hashemi F[1], Zabolian AH[5]

1. Young Researcher and Elite Club, Islamic Azad University, Kazerun, Iran
2. Department of Biology, Faculty of Science, Islamic Azad University, Shahrekord, Iran
3. Faculty of Veterinary Medicine, Islamic Azad University, Kazerun, Iran
4. Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran
5. Young Researcher and Elite Club, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran.
Email: fzr2000_0007@gmail.com

Background: HeLa is the most widely used model cell line for studying human cellular and molecular biology. Cervical cancer is the third most common cancer and the fourth cause of death due to cancer among women in the world, accounting for more than 80% of deaths from this cancer type in developing countries. Polyphenolic compounds were used as natural anticancer agents due to anti-inflammatory, anti-oxidant, anti-mutagenic and inhibitory effects and tumor growth. The aim of this study is to investigate the effect of trans cinnamic acid on Hela cancer cell line.

Materials and Methods: Hela cells were seeded and treated with 5, 25, 50 and 100 mM concentrations of trans cinnamic acid for 1, 3 and 5 days. Cell viability was evaluated on certain days after treatment. We used MTT assay to confirm cell viability. The absorbance of plates was measured at 570 nm using an ELISA reader. Also, Acridin Orange (AO) staining was used to detect apoptosis.

Results: We observed a significant change in cell viability of treated cells with tran cinnamic acid. AO staining confirmed apoptotic morphology in treated cells.

Conclusion: Therefore, we conclude that trans cinnamic acid had an anticancer effect on Hela cells.

Keywords: HeLa, Acridin Orange,Trans Cinnamic Acid, MTT Assay

Ps-26: Investigation for The Prognostic Value of Echocardiographic Indices and Proposing a Reliable Cut-Off For E/Em (E, Peak Early Trans-Mitral Velocity; Em, Early Diastolic Mitral Annular Velocity) as An Independent Prognostic Factor

Dehghan AR

Mashhad University of Medical Sciences, Mashhad, Iran
Email: armdehghan@gmail.com

Background: The aim of our study was to evaluate the prognostic value of TDI factors and propose a reliable cut-off for E/Em (E, peak early trans-mitral filling velocity; Em, early diastolic mitral annular velocity) as a prognostic TDI index

Materials and Methods: By purposive sampling, 100 HFpEF patients, according to Framingham criteria and ejection fraction (EF) >45%, pro-B type Natriuretic Peptide>500pg/ml, and diastolic dysfunction were included in our longitudinal study. Patients suffered from atrial fibrillation, myocardial infarction, valvular disorders, congenital heart disease, EF<45%, previous lung, liver, and kidney disease were excluded from the study. At admission, all patients underwent trans-esophageal TDI. TDI parameters including E/EM, deceleration time (DT), E/A (A, late trans-mitral filling velocity), left ventricular end-diastolic time (LVEDD) and LAVI (left atrium volume index) were measured. Also, underlying diseases, including Diabetes mellitus, hypertension, smoking, dyslipidemia, and coronary artery

disease were taken into consideration. After a 6 month follow-up (from February 2015 to July 2015), in order to evaluate prognosis, the patients were classified into two groups (with or without morbidity). Morbidity was defined as rehospitalization, need for inotrope, and cardiorenal syndrome occurrence. For data analyses, we used SPSS 16.0. Regression test, Chi-square, and student t-test were performed. We also designed ROC curve to show the specificity and sensitivity. The best cut-off for parameters were measured by Youden index.
Results: At the end of the study, 24 cases showed morbidity. None of the patients died during our study period. Higher mean values of E/Em and E/A correlate with a higher incidence of morbidity (all parameters, P<0.001). Also lower mean values of DT associate with morbidity (P<0.001). There was not any prognostic value for LVEDD, LAVI, and underlying diseases (all parameters, P>0.05). Regression test presented E/Em and DT as independent factors in HFpEF prognosis. At the cut-off of 13.5, E/Em was 97.1% sensitive and 55.3% specific.
Conclusion: At the cut-off of 13.5, we found E/Em as a sensitive, specific, and independent prognostic parameter for HFpEF. Also, we found DT as an independent prognostic TDI index.
Keywords: HFpEF, Prognosis, Echocardiography, E/Em, DT

Ps-27: Applying Steady Flow to A Mesanchymal Stem Cell for Differntiating into Cardiomyocyte: A Simulation Study

Ebad M*, Vahidi B

Division of Biomedical Engineering, Department of Life Science Engineering, Faculty of New
Sciences and Technologies, University of Tehran, Tehran, Iran
Email: bahman.vahidi@ut.ac.ir

Background: Presently, surgical interventions for the treatment of cardiovascular diseases encounter restrictions such as lack of donor organs for transplant, limited strength of transplanted organs and the need of prescription after transplantation. Tissue engineering is a new field to overcome these limitations. Stem cells are the optimal cell source for heart tissue engineering due to their ability to self-repair and differentiating to cardiomyocytes. Blood flow is one of the most important mechanical stimulations for differentiating of stem cells, adjustment of cardiovascular function and homeostasis.
Materials and Methods: Simulation of fluid-solid interaction was accomplished to investigate the application of steady flow on a single mesenchymal stem cell adhering to a fibronectin membrane in a perfusion bioreactor. Laminar flow was applied for simplification. the fluid was considered water and the cell had elastic material properties. The effect of flow variables on stem cell behavior in order to differentiate into cardiomyocyte cells and its mechanobiology was analyzed. This simulation has been done by ANSYS software.
Results: The maximum von mises stress achieved on the cell membrane is about 5-10 dyn/cm^2. According to previous researches, in this stress, cell response of cardiac markers, such as MEF2C and sarcomeric α-actinin, increase a significant amount.
Conclusion: In this study, by applying flow with the velocity of 0.33 mm/s, the amount of stress is within the range of 5-10 dyn/cm^2 by considering a three-dimensional cell model comprising of nucleus and cytoplasm. Corresponding to studies beforehand, with in the mentioned range of stresses, the fate of the stem cell may be distinguished to cardiomyocytes. It is suggested for future studies that the effect of pulsatile flow on the maximum von mises stress should be considered.
Keywords: Mesenchymal Stem Cell, Mechanotransduction, Fluid-Solid Interaction, Cell Mechanics

Ps-28: The Impact of Mir-302/367 Cluster on Pluripotency and Dopaminergic Differentiation of Human Adipose Tissue-Derived Stem Cells

Faghih H*, Javeri A, Fakhr Taha M, Shamsara M

Department of Stem Cells and Regenerative Medicine, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: arashj@nigeb.ac.ir

Background: Adipose tissue-derived stem cells (ADSCs) secrete angiogenic, anti-inflammatory, immunomodulatory and neuroprotective factors. Also, several studies have documented neural and dopaminergic differentiation of the ADSCs which raises the hope of using these cells for the treatment of neurodegenerative disorders including Parkinson's disease. However, for clinical application, highly efficient techniques are required to generate a purified population of dopaminergic neurons. One solution may be reprogramming of the ADSCs towards a more pluripotent state by different strategies. Here, we used miR-302/367 cluster to improve pluripotency and dopaminergic differentiation potential of human ADSCs.
Materials and Methods: Third-passaged ADSCs were transfected with the pTD.CMV-hsa-miR-302bcad/367-EF1-GFP-T2A-Puro (System Biosciences, USA) using a Neon Transfection system (Invitrogen, Thermo Fisher Scientific, USA). After three weeks, the ADSCs were assessed for the expression of pluripotency genes. Also, dopaminergic differentiation of the control and transfected ADSCs was induced by a growth factor cocktail consisting of SHH, FGF-8, b-FGF and BDNF and analysed by RT-PCR, quantitative real-time PCR, immunocytochemistry and western blot.
Results: Transfection of the ADSCs with miR-302/367 cluster upregulated the expression of OCT4, NANOG, SOX2, REX1 and NODAL mRNAs. Both the control and miR-302/367-transfected cells were successfully differentiated into dopaminergic neurons and expressed some genes associated with dopaminergic differentiation, including EN1, GLI1, NURR1, TH, and GIRK2 mRNAs and TUJ1 and TH proteins while TH and GIRK2 expressions were upregulated.
Conclusion: These findings show that miR-302/367 cluster reprograms human ADSCs towards a more pluripotent state and improves dopaminergic differentiation potential of the ADSCs which may be in favour of future clinical application of these cells.
Keywords: ADSC, miR302/367, Dopaminergic Differentiation, Pluripotency

Ps-29: Comparing Histone Modification for Transcription Factor and Chemical Induction of Fibroblast into Cardiomyocyte

Falsafi N*, Fallahi H

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

Background: The potential of reprogramming of mammalian heart is limited, Majority of them fail to recover during injury

and disease. Hence, reprogramming of cardiac cell from different kind of stem and non-stem cells has a great potential for basic studies and therapeutic application and can be carry out as innovative strategies for replacing cardiac cells. Different steps were developed to regulate the expression of genes involved in reprogramming of cardiac cells. One of the steps in regulating the gene expression in cells undergo reprogramming either by a number of chemicals or transcription factors is histone modification. Modification in histones including methylation, acetylation etc., play a crucial role in conversion of cardiac cells which is poorly investigated. In this study, we identified histone modification for two type of induction in response to transcription factors and chemicals. For this goal, we have used two transcriptome datasets from direct conversion to cardiac cells by TFs and chemicals to identify the key modification of histones in either of studies and then comprising similarities/differences.
Materials and Methods: In this study, differentially expressed genes with fold change equal or above 1.5 were isolated from total gene expression, using R language program for both studies. Then, histone modification extracted by Enrichr online tools. Plus, the modifications of histone with highest number of score in regulatory network were extracted. Finally, we compared the modification for similarities/differences in both studies.
Results: Network Analysis have showed the most important differentially expressed genes and their histone modifications during early stage of maturation of induced cardiomyocyte. Besides, we have introduced key histone modifications during this conversion, based on type of induction (chemical or TF).
Conclusion: Our finding could be helpful to achieve regulatory elements involved in maturation pathway due to their type of induction. Also, key histone modifications would be useful in generating of more efficient induced cardiomyocytes for therapeutic applications.
Keywords: Direct Conversion, Histone Modification, Network Analysis

Ps-30: Evaluation of Major Histocompatibility Complex Genes Expression in Somatic Cell Nuclear Transfer Embryonic Stem Cells

Farahi S*, Farifteh F, Salehi M

Department of Biotechnology, Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Msalehi78@gmail.com

Background: Embryonic stem cells (ESCs) have the potential to differentiate into almost all cell types providing a promising cell source in regenerative medicine. The low efficiency of somatic cell nuclear transfer (SCNT), the technique to achieve blastocyst for ESC generation, has been improved by trichostatin A (TSA) a histone deacetylase (HDACi) inhibitor, which change pluripotent mediated genes expression during reprogramming. Since TSA changes MHC molecules expression on tumor cells which cause immune responses against them. Hence, this study aims to determine whether TSA affects MHC expression levels of ESCs.
Materials and Methods: Toward this goal, we generated SCNT-ESCs in the presence and absence of TSA. IVF-ESCs was established as the control group. ESCs were confirmed by alkaline phosphatase assay and immunocytochemistry (ICC). The expression level of MHC molecules (i.e. H2Kb, H2Kd, H2Dd, Qa-1) for both blastocyst and ESCs generated by two techniques were examined by qRT-PCR.
Results: Our results revealed that the expression levels of H2Kb, H2Kd, H2Dd, and Qa-1 (MHCI) in SCNT-ESCs increased in the presence of TSA compared to control group but they were not statistically significant. In contrast, these genes were significantly downregulated in the TSA negative group. We observed increased expression level of MHCI molecules in both blastocyst-derived TSA positive and negative SCNT.
Conclusion: Although TSA plays a key role in the developmental rate of embryos as well as establishment of ESC lines after SCNT, behind of its negative effect on MHC expression we suggest eliminating TSA treatment after SCNT, so that we could gain better NT-ESC.
Keywords: Somatic Cell Nuclear Transfer, Major Histocompatibility Complex, Stem Cell

Ps-31: Differentiation Potency of Bone Marrow C-Kit+ Hematopoietic Progenitor Cells towards Natural Killer Cell Line

Farahzadi R[1*], Fathi E[2]

1. Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran, Iran
Email: farahzadir@tbzmed.ac.ir

Background: As previously indicated, natural killer (NK) cells play a key role in the elimination of compromised host cells, such as tumor or virus-infected cells. The ability of NK cells to kill without prior sensitization has made them attractive for cell therapy. Notably, the use of NK cells for cell therapy relies on the availability of a great number of NK cells with optimal cytotoxic activity. Obtaining a large number of NK cells is an important, although difficult task that underlies the most significant challenge to the development of successful NK cell adoptive transfer protocols. NK cells can be differentiated from stem cells and can be used directly for immunotherapy or after a short- or long-term expansion *in vitro* with interleukins such as IL-2, IL-7 and IL-15. Since access to cord blood cells is less feasible, another approach is the generation of NK cells from stem cells especially hematopoietic stem cells. Previous *in vitro* studies have shown that using specific fundamental factors, bone marrow lineage hematopoietic stem cells can differentiate into the NK cells.
Materials and Methods: In this experimental study, mononuclear cells (MNCs) were collected by Ficoll-Hypaque gradient centrifugation and c-kit+ cells were enriched by MACS method. In the following, c-kit+ cells were treated in the presence of NK differential factors such as SCF (kit ligand), FLT3 and IL-15 for 21 days. At the end of treatment period, differentiated cells were collected and subjected to flow cytometry for detecting the NK cell markers.
Results: The results of this study showed that the c-kit+ differentiated cells were positive for NK cells marker CD56 (70.5%).
Conclusion: It was concluded that the c-kit+ cells as hematopoietic progenitor cells could differentiate to NK cells. In the following of obtaining satisfactory results from this project, it is recommended to apply these results to the animal model and eventually conduct large scale clinical trial in future to use these cells to large number of patients in terms to prove its efficacy more authentic way.
Keywords: C-kit+ Hematopoietic Progenitor Cells, NK Cells,

Differentiation Potency

Ps-32: Fabrication and Characterization of Nanocomposite Membrane Based on GelMA-Silk Nanofibrils as A Bioengineered Neo-Corneal Stroma

Farasatkia A[1*], Kharaziha M[1], Ashrafizadeh F[1], Salehi S[2]
1 Department of Materials Engineering, Isfahan University of Technology, Isfahan, Iran
2 Institute of Anatomy, Essen University Hospital, University of Duisburg-Essen, Germany
Email: asalfarasatkia@yahoo.com

Background: Due to the increasing incidence of diseases and injuries to the cornea of the human eye and the limitation of this sensitive tissue to receive medical treatments or ocular implants, as well as corneal transplantation problems, cornea reconstruction is desirable.
Materials and Methods: Nanocomposite membrane of GelMa-silk nano fibroin is prepared. The silk nanofiber reinforcement increases both transparency and strength of the hydrogels improving handling and integrity.
The wettability, transparency and mechanical properties of the composites with different volume ratios of GelMa/SNF were measured.
Conclusion: According to research on corneal stromal tissue engineering, the construction of bi-sticky scaffolds with sufficient transparency and the ability to accelerate tissue regeneration is prepared and characterized.
Keywords: GelMA, Silk Nanofibrill, Nanocomposite, Stroma, Corneal Tissue Engineering

Ps-33: Role of Antioxidant Components on Formation of 3D Human Embryonic Stem Cell-Derived Organoids

Farasatpour Z[*], Karamali F, Savoj S, Nasr Esfahani MH

Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: Frkaramali60@gmail.com

Background: According to Eiraku protocol, the human embryonic stem cells (hESCs, RH6 cell line) colonies were dissociated to single cells using accumax enzyme and plated in U-shape low adherent 98 well plate.
Materials and Methods: In addition of Rock inhibitor, Y-27632 which was considered as control group, different antioxidant components including Tempol, Alpha lipoic Acid, Ascorbic Acid, Quercetin and N-acetylcysteine with different concentrations were used.. Two days later, the cells survival was assessed under an inverted microscope. Also, the aggregate size was analyzed one week later.
Results: Our data expressed that high concentrations of Quercetin and tempol induced the cell death of hESCs, even in the presence of Y-27632. Whereas the lower concentrations of these components play a protective role in dissociated HESCs. The measurement of aggregate size confirmed the protective effect of different antioxidant components to increase the quality of hESC derived organoids.
Conclusion: Taking advantage of the original retina organoid protocol, we investigated the effects of antioxidant components after dissociation of human embryonic stem cells. Our results showed that although some high concentrations of antioxidant revealed some deleterious effects on cell viability, in almost all optimal concentration of antioxidants, the survival was improved comparing control group. Therefore, one of the future efforts will be to apply and optimize the antioxidant component(s) to the human retinal organoid system.
Keywords: Antioxidant, Organoid, Embryonic Stem Cell

Ps-34: To Study Tauopathy Molecular Mechanism Upon Diabetes

Farhadi A[1, 2*], Shahpasand K[2], Tahamtani Y[3]

1. Department of Developmental 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 Brain and Cognitive Science, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: -Yaserta@yahoo.com

Background: Diabetes mellitus (DM) is a metabolic disorder in which various organs are being affected. In particular, neural cells severely respond to the stress conditions caused by DM, resulting in neurodegeneration. There are two major pathological hallmarks playing part in the neurodegeneration including hyperphosphorylated tau (P-tau) and amyloid β plaques. Tau is a microtubule associated protein, which is moderately phosphorylated under physiological conditions and is hyperphosphorylated upon abnormal situations. It is well-documented that diabetes can cause tau phosphorylation on different sites. Anyway, it's not clear that which tau phosphorylation site plays the critical role in driving neurotoxicity and tauopathy upon Diabetes. Recently, It has been shown that phosphorylated tau at T231 exists in the two distinct cis and trans conformations in which that cis p-tau is extremely neurotoxic and can be accumulated in neurons cultured under nutrition depletion. These observations led us to hypothesis that DM can induce cis p-tau in type 1 and 2 diabetic mouse models.
Materials and Methods: 70 mg/kg Alloxan was used to induce hyperglycemia in mice models. We performed immunostaining with cis p-tau monoclonal antibody after hyperglycemia induction.
Results: We observed cis p-tau at early stages of hyperglycemia in in vivo and *in vitro* models. Interestingly we have detected cis p-tau in specific brain regions like corpus callosum and brain stem. Moreover, GABAergic and Glutamatergic neurons were significantly positive for cis p-tau compare to other neurons.
Conclusion: Our findings demonstrate that Neurodegeneration, resulted by Diabetes, is being driven through cis p-tau and thus, prospective therapies against cis p-tau could be applicable for Diabetes. The results open new windows toward understanding the molecular mechanism of neurodegeneration upon DM.
Keywords: Neurodegeneration, Tauopathy, cis p-tau, Immunotherapy, Diabetes Mellitus

Ps-35: L-Carnitine Contribute to Promote Cardiogenesis of C-Kit+ Progenitor Cells-Derived Bone Marrow
Fathi E[1*], Farahzadi R[2], Vietor I[3], Javanmardi S[1]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Division of Cell Biology, Biocenter, Medical University Innsbruck,

Innsbruck, Austria
Corresponding Email: ez.fathi@tabrizu.ac.ir

Background: C-kit+ Bone marrow progenitor cells have recently gained much attention due to the therapeutic effects following their administration into the infarcted heart as evidenced by animal studies and by a recent clinical trial. However, injecting these cells in the heart is associated with poor differentiation into specialized cardiac cell types and with rapid death of the engrafted cells. With the ultimate goal of advancing cardiac stem cell therapy, we sought to facilitate the differentiation of c-kit+ Bone marrow progenitor cells into cardiac cell types (e.g. cardiomyocytes, smooth muscle cells, endothelial cells and cardiac fibroblasts) by overexpressing selected cardiac transcription factors *in vitro*. On the other hand, the effective role of L-carnitine as strong and effective antioxidant in improved cardiac function has been proven.
Materials and Methods: For this purpose, mononuclear cells were isolated from rat bone marrow and c-kit+ (CD117) cells were separated with MACs. In the following, c-kit+ cells were treated with L-carnitine for 7, 14 and 21 days and gene expression of cardiac TFs, including Gata4 and TBX5 were evaluated by Real time PCR.
Results: It was shown that L-carnitine cause to significant increase in gene expression of Gata4 and TBX5. In detail, it was found that L-carnitine promoted the cardiogenesis of c-kit+ progenitor cells.
Conclusion: It was concluded that L-carnitine could be used for facilitating the differentiation of c-kit+ Bone marrow progenitor cells into cardiac cell types with the aim of cell-based therapy.
Keywords: L-carnitine, C-kit+ Progenitor Cells, Cardiogenesis

Ps-36: Mesenchymal Stem Cells Induced Early Apoptosis in B65-Neurogenic Differentiated Cells as *In Vitro* Cell Line Model for Alzheimers Disease

Fathi E[1*], Farahzadi R[2], Vietor I[3]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3. Division of Cell Biology, Biocenter, Medical University Innsbruck, Innrain 80-82, A-6020, Innsbruck, Austria
Email: ez.fathi@tabrizu.ac.ir

Background: Mesenchymal stem cells (MSCs) as undifferentiated multipotent cells are of particular interest due their potential clinical use in regenerative medicine. Stem cell-based therapies cast a new hope for neurodegenerative disease such as Alzheimer's disease (AD) treatment as a replacement or regeneration strategy. Neurodegenerative diseases are groups of acute or/and chronic diseases that depending on operating condition cause to loss of neuroglia as well as neural cells. There is not absolute treatment due to the complete lack of understanding of the factors and conditions involved in them. Understanding the cellular mechanisms involved in Alzheimer's patients experience memory impairment is essential to design and determine the effectiveness of new drugs and therapies. The aim of this study is to explore the influence of secreted cytokines of MSCs on apoptosis of B65-neurogenic differentiated cell line as *in vitro* cell line model for AD.
Materials and Methods: For this purpose, MSCs were isolated from bone marrow and co-cultured with B65-neurogenic differentiated cell (1:10) for 5 days. At the end of co-culture period, B65 differentiated cells were collected and subjected to Annexin/PI assessment.
Results: It was shown that MSCs cause to significant induction of early apoptosis in B65-neurogenic differentiated cell. In detail, it was found that 37.3% of the cells were in early apoptotic stage (Annexin+, PI-), which was 2.91 times higher than that of the control group (12.8%).
Conclusion: In this study, we reported that MSCS could be used for apoptosing B65-neurogenic differentiated cell as model in cell-based therapy for AD.
Keywords: Mesenchymal Stem Cell, B65-Neurogenic Differentiated Cells, Alzheimers Disease

Ps-37: Cytotoxic and Antioxidant Activity of N-Hexane Fraction of Bandotan Herb (Ageratum Conyzoides L.) and Doxorubicin as Co-Chemotherapeutic Agent in Breast Cancer Cells Line by *In Vitro* and in Silico Assay

Febriansah R[*], Lubis W A

School of Pharmacy, Faculty of Medicine and Health Sciences, University of Muhammadiyah, Yogyakarta, Indonesia
Email: briansyah_rifki@yahoo.com

Background: Breast cancer is one of the most common cancers that causes the highest morbidity and mortality in humans. Some anticancer medicines are not efficient and also have many side effects. Based on the previous research showed bandotan herb extract contained several compounds which potentially have anti-microbial, anti-inflammatory and anti-cancer effects. This study aimed to analyze the cytotoxic activity and co-chemotherapy effect of n-hexane fraction of Bandotan (NFB) combined with doxorubicin in MCF-7 breast cancer cells line by *in vitro* assay and study the molecular mechanism of the active component NFB by in silico assay, with HER-2 as protein target.
Materials and Methods: Antioxidant activity test of NFB measured by DPPH method and cytotoxicity assay of NFB performed with MTT assay method was carried out by molecular docking method between ageratochromene dimer (compound marker of bandotan) on HER-2 protein. NFB also tested by flowcytometer to know the effect of the extract in cell cycle inhibition.
Results: Based on the research showed that 1 kg of Ageratum conyzoides L. obtained 1.5 gram NFB concentrated exctract which would then be utilized in antioxidant and cytotoxic test. The docking molecular method results showed that NFB has potential cytotoxic agent mediated by the ability of ageratochromene dimer inhibited HER-2 protein targets with score affinity of -6.2 kcal/mol and -8.5 kcal/mol with doxorubicin as comparison with affinity of -6.9 kcal/mol on HER-2. Antioxidant activity of NFB showed the IC50 value was 493 µg/ml. The NFB had potency as citotoxic activity with IC50 value of NFB 306 µg/ml to inhibit the growth of MCF-7 cells line and showed synergistic result in combination with doxorubicin as chemotherapy with Combination Index (CI) being between 0.53-0.90. Based on flowcytometry analysis showed that NFB could inhibit the S phase of the MCF-7 cells line.
Conclusion: From the result obtained it could be concluded that the NFB has weak potential as antioxidant activity and synergistic effect as co-chemotherapy with doxorubicin that inhibit in S phase in MCF-7 breast cancer cells line based on *in vitro*

and in silico assay.

Keywords: Ageratum Conyzoides L, Antioxidant, Co-Chemotherapy Effect, Molecular Docking, MCF-7 Cells Line

Ps-38: Identification of Five Top Micrornas Involved in The Middle of Hepatic Differentiation of Mesenchymal Stem Cells

Ghaderi Gandomani M[1*], Sahebghadam Lotfi A[2], Kordi Tamandani DM[1]

1. Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran
2. Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: lotfi_ab@modares.ac.ir

Background: Aim: Human Adipose Derived Mesenchymal Stem Cells (hADSCs) are capable to self-renew and differentiate into hepatocytes. MicroRNAs (miRNAs) are a class of short, non-coding RNAs that regulate gene expression post-transcriptionally. Although miRNAs have emerged as key regulators of differentiation and cell fate decisions, a few studies have been reported about the complex expression patterns of miRNAs during differentiation of hADSCs into hepatocyte and the role of miRNAs in the regulation of this process. The aim of this study was to identify specific miRNAs involved in the middle stage of hepatic differentiation of hADSCs.
Materials and Methods: hADSCs were differentiated to hepatocytes by a two-step protocol. After characterization of hepatic differentiation of hADSCs by biochemical, morphological and molecular methods, miRNA expression was profiled in differentiated hADSCs at days 7 and 14 using miRNA microarray analysis.
Results: Among 321 miRNAs significantly differentially expressed between the two samples, 135 and 186 miRNAs were significantly up-regulated and down-regulated during middle stage of differentiation, respectively. Top 5 ranking miRNAs in volume with significant cut-off, $|FC|\geq 2$ or $|FC|\leq 2$, were hsa-miR-222-3p, hsa-miR-23b-3p, hsa-let-7e-5p, hsa-miR-103a-3p and hsa-let-7d-5p.
Conclusion: The results indicate that different kinds of miRNAs may function during the middle stage of differentiation of hADSCs into hepatocytes.

Keywords: miRNA, Hepatocyte, Differentiation, Mesenchymal Stem Cell, Microarray

Ps-39: Nitrogen-Containing Plasma Polymer Films Deposited by PECVD Process on Expression of Type X Collagen

Ghafouri S[1, 2, 3*], Abdijahed S[3], Farivar Sh[3], Shokri B[1, 2]

1. Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran
2. Department of Physics, Shahid Beheshti University, Tehran, Iran
3. Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran
4. Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran
Email: plasma313@gmail.com

Background: Nowadays, nitrogen-containing plasma polymers, due to their cell adhesion and influence on the response of mesenchymal stem cells (MSCs) are of great interest. The major problem of cartilage tissue engineering is that MSCs from osteoarthritis (OA) patients express high levels of type X collagen. This problem could be solved by these films. In this study, low-pressure plasma co-polymerization using binary gas mixtures of acetylene and nitrogen is investigated to deposit Nitrogen containing plasma-polymerized (L-PPA: N) coatings in radio frequency (RF: 13.56 MHz). In this research, we tried to decrease the concentration of amine groups to evaluate their effect on the expression of collagen X. To study the chemical bonding states and surface potential of the deposited films, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), and zeta potential analysis was used. Finally, the response of mesenchymal stem cells under the influence of these films and the expression of type X collagen were examined by Real-time- PCR.
Materials and Methods: The preparation of Nitrogen-containing plasma-polymerized (L-PPA: N) coatings on surfaces were performed in the reactor operating by an RF of 13.56 MHz in a parallel plate capacitively coupled configuration, which was used for generation of plasma from a mixture of nitrogen (99.9995% purity) with acetylene as a monomer (99.6% purity). The plasma deposition time, operating pressure and the gas flow ratio R (FN2/FC2H2) were kept constant at 10 min, 25 mTorr and 2, respectively. The ATR- FTIR spectra were recorded using a Bruker Tensor 27 in the spectral range of 4000–400 cm−1with the resolution of 5 cm−1and 100scans. The measurement of zeta potential of the samples was made 3 hours after plasma deposition using an Electrokinetic Analyzer (EKA Sur PASS–type A, Anton Paar GmbH, Graz, Austria). For this measurement, KCl 0.001M was used as titrator. Mesenchymal stem cells were seeded on L-PPA: N surface. The RNA extraction was done after 4, 7 and 14 days and the expression of Col X gene was evaluated. One-way ANOVA test was applied to analyze the results statistically.
Results: The Zeta potential measurement indicated that the surface potential of the typical L-PPA: N surface is -30.13. Since the presence of anions on surfaces caused negative zeta potential, the presence of more carboxyl groups on the surface was verified. According to the spectrum, three bonds were indicated: I. 3000 - 2800 cm-1 C-H stretch; II. 1800 - 1500 cm-1 C=C, C=N stretch; and III. 1500 - 1300 cm-1 CH2, CH3 bending. Band I comprises a convolution of contributions due to aliphatic CH groups. The assignment of band II is somewhat speculative, the likeliest contributions being C=C or C=N stretching modes. Finally, band III is attributed to disordered highly-ranched aliphatic chains. The results of the Real-time q-PCR indicated that Col X gene expression significantly suppressed in 7 days. Again, in the day of 14th, the data showed a significant increase in the expression of this gene which means that the biological activity of the films is lost within about ten days.
Conclusion: It was probable that the significant potential difference across the cell membrane and L-PPA: N surface caused attraction between them. FTIR results showed a remarkable amount of carboxyl group and a negligible amount of amine group. It is proved that the surface containing a high amount of nitrogen moieties might lead to long-standing suppression of collagen expression. Since, cellular adhesion has a strong effect on gene expression and also on collagen expression and differentiation- functional group on L-PPA: N surface influences cellular adhesion into the extracellular matrix (ECM). Accordingly, it could be concluded that the functional group has a substantial impact on gene expression and differentiation. Regarding the results of the real-time qPCR assay of the PPA: N surface, absence of suppression in the long-time period could

be most probably assigned to amine groups.
Keywords: Thin Films, Low- Pressure Radio Frequency Plasma, Cartilage, Type X Collagen

Ps-40: Gene regulatory Network of Osteosarcoma

Ghamari N*, Radak M, Fallahi H

Department of Biology, Razi University of Medical Sciences, kermanshah, Iran
Email: nakisa.ghamari1414@gmail.com

Background: Osteosarcoma is a common primary malignancy of bone in children and young adults. Sporadic mutations are the most important factor in causing osteosarcoma, Especially mutations in tumor suppressor genes. In this research, we analyzed gene regulatory network of osteosarcoma.
Materials and Methods: We used GSE69524 dataset (GPL11028 platform), which information is freely available in the NCBI database. Dataset was analyzed using limma package embedded in GEO2R tool of NCBI to determine significantly, differentially expressed genes (DEGs) with a P-value less than 0.005 in osteosarcoma cells against normal human osteoblast. After that, selected the genes that have ± 0.6 of log2 Fold change. Next, we predict transcription factors of differentially expressed genes in Enricher site with ChEA2016. At last, we used Cytoscape 3.4.0 and Gephi 0.9.2 softwares for constructed and analyzed a network of differentially expressed transcription factors (DE-TF).
Results: The result showed that there are 12 differentially expressed transcription factors (DE-TF). In osteosarcoma cancer, 4 of transcription factors were down-regulated while 8 were upregulated. Furthermore, among DE-TFs that have been, downregulated HNF4A has the highest outdegree and CHD1 has the highest outdegree between up-regulated DE-TFs.
Conclusion: The results and interpretation of this information and the molecular differences between normal human osteoblast and osteosarcoma cells show us the path to find better treatment of this cancer, especially molecular therapies, with fewer complications.
Keywords: Osteosarcoma, Differentially Expressed Transcription Factors, Osteoblast

Ps-41: TGF-B Signaling Play A Critical Role in De Novo Generation of $CD^{4+}CD^{25+}$ CD127dim Cells from Naïve T Cells in Comparison with Exogenous Foxp3 Transduction

Haddadi M[1, 2*], Hajizadeh-Saffar E[2], Basiri M[3], Negahdari B[1], Baharvand H[3]

1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Science, Tehran, Iran
2. Department of Regenerative Medicine, 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
Email: Hajizadeh.ehs@royaninstitute.org

Background: T-regulatory cell (Treg) therapy represents the immunological peripheral tolerance in allogeneic transplantation and autoimmune diseases. Treg can be derived from progenitor naïve CD4+ T cells through TGF-β treatment and/or exogenous Foxp3 transduction, while they show plasticity toward TH17 phenotype in inflammatory condition. In this study, we determine the in-vitro stability of Treg phenotype and also suppressive activity both in TGF-β-dependent Treg differentiation and Treg differentiated with exogenous Foxp3 transduction.
Materials and Methods: Tregs were made by TGF-B and Foxp3 transduction from naïve CD4+ T cells in a normal and inflammatory milieu during 10 days. The inflamed condition presented by IL-6 and SR1555 small molecule was used to avoid trans-differentiation toward TH17. Treg phenotype (CD4+ CD25+ CD127dim) was determined by flow cytometry and CFSE was used to measure the suppressive activity. The concentration of IL-17, TGF-β, IL-10 and IFN-γ was determined by ELISA.
The yield of Treg differentiation in the presence of TGF-β was significantly higher than Foxp3-transduced group. In the presence of IL-6 and SR1555, cultured TGF-β-dependent Tregs maintained stable phenotype and they have shown higher suppressive activity than Foxp3-transduced groups. We had an increase in suppression activity when adding 10μg/ml SR1555. Low suppressive activity was observed with loss of Treg phenotype in the presence of IL-6 in Foxp3-transduced groups in comparison with TGF-β group in days 5.
Conclusion: Our finding indicated that to de novo generation of Treg, TGF-β treatment is more applicable than exogenous Foxp3 transduction and TGF-β signaling is important to maintain stability of Treg phenotype and its suppressive activity.
Acknowledge: An encouragement grant has been awarded to this study from Royan Stem Cell Technology Company, Tehran, Iran.
Keywords: Treg Generation, Naive T Cell into Treg, Treg/Th17 Imbalance, Treg Trans-Differentiation, Treg Therapy

Ps-42: Autophagy A Key Target for Induction of Apoptosis in Acute Promyelocytic Leukemia

Haghi A[1*], Salemi M[1], Mohammadikian M[1], Fakhimahmadi A[2], Nikbakht M[1], Moosavi MA[2], Mohammadi S[1], Rahmati M[3]

1. Tehran University of Medical Sciences, Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Molecular Medicine, Institute of Medical Biotechnology, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran
3. Cancer Biology Research Center, Tehran University of Medical Sciences, Tehran, Iran
Email: atousahaghi@gmail.com

Background: Treatment of acute promyelocytic leukemia (APL) a subtype of acute myeloid leukemia (AML) develops as a result of a series of genetic mutations in a hematopoietic precursor cell. The incapability to undergo apoptosis is a key mechanism of multidrug resistance in AML, and the analysis of autophagy and apoptotic factors may demonstrate a significant prognostic tool to predict the outcome. The pro-survival function of autophagy is known to be adaptive, but in the context of cancer, is significantly abnormal. Resistance cell death owing to activation of autophagy prosurvival in cancer cells. In this study, we evaluate the level of LC3II and caspase3 after treatment NB4 cell line by Arsenic trioxide, All-trans retinoic acid, 3-MA, BafA1 and HCQ.
Materials and Methods: We studied *in vitro* effects of Arsenic trioxide, All-trans retinoic acid, 3-MA, BafA1 and HCQ on hu-

man leukemia cell line (NB4) in a dose-dependent and time-dependent manner which investigated through cell proliferation assay in both single and combination doses. Next the rate of apoptosis (annexin V FITC assay) and cell cycle arrest measured by flow cytometry. The development of acidic vesicular organelles (AVOs) specified by acridine orange (AO) staining of the cells, then Photos were obtained with a fluorescence microscope. In addition, we evaluate the level of LC3II protein by western blot. And finally, the level caspase3 mRNA expression was evaluated via real-time PCR.
Results: The data show that a combination of ATO (1μM) and ATRA (700nM) and HCQ effectively induced apoptosis and decreased leukemic cell proliferation. Real-time PCR analysis indicated that mRNA expression of Cas3 was increased which was accompanied by accumulation of LC3II protein.
Conclusion: These *in vitro* studies imply that ATO/ATRA/HCQ has a direct antileukemic effect by inhibiting autophagy factors.
Keywords: Leukemia, Apoptosis, Autophagy

Ps-43: Effect of Cholestasis Syndrome on Proliferation Potential of Spermatogonial Stem Cells

Hasani Fard AH*, Mohseni Kouchesfahani H, Jalali H

Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
Email: hfdamir@yahoo.com

Background: Cholestasis is a pathophysiological condition that is occurred due to various reasons such as infection, autoimmunity, metabolic disorders or genetic factors. Histological studies have shown that cholestasis reduces proliferation, causes apoptosis, and prevents the maturation of germ cells. Spermatogonial stem cells (SSCs) are essential for self-renewal and differentiation during spermatogenesis. In current study we conducted a study to investigate the effect of cholestasis on the proliferation and viability of SSCs in the *in vitro* condition.
Materials and Methods: Obstructive cholestasis was inducted in male Wistar rats by ligating the common bile duct. SSCs were isolated from healthy and cholestatic rats and the duration of colony appearance, the diameter of clones and the viability of SSCs were determined in culture medium. Briefly, the clones were fixed using paraformaldehyde 4% and subsequently, the number and diameter of the SSCs were determined under a microscope. To determine cell viability MTT assay was performed.
Results: SSCs clones were observed in the control group after 2 days while they were appeared in the cholestatic group after 4 days. The results indicated that SSC had more proliferation in the control group within two weeks. There was also a significant difference between the diameter of the SSCs clones in the cholestasis group compared to the control group and SSCs clones in the control group have been wider in two weeks. There was no significant difference in the survival of SSCs between two groups within two weeks.
Conclusion: As expected on the basis of tissue studies, comparison of proliferation and viability rate of SSCs between healthy and cholestatic rats indicated that the number of stem cells in damaged animals decreases dramatically, and affects their ability to multiply in the medium. The current study shows one of the important side effects of cholestasis on testicular tissue and consequent infertility problems in men.
Keywords: Testis, Spermatognial Stem Cell, Proliferation, Cholestasis, Fertility

Ps-44: Long Term Maintenance of Functional Primary Human Hepatocytes in Organoid Culture Using Natural Microparticles

Heydari Z[1*], Zarkesh L[2], Ghanian MH[2], Baharvand H[1], Vosough M[1, 3]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Tehran, Iran
2. Department of Cell Engineering, Cell Science Research Center, Tehran, Iran
3. Department of Regenerative Medicine, Cell Science Research Center, Tehran, Iran
Email: masvos@Royaninstitute.org

Background: To discover new drugs in clinical trials, extensive preclinical testing has to be done. Through nine developed compounds one of them get approved by the FDA. Animal models and *in vitro* models are both currently used to investigate pharmacodynamics and pharmacokinetics of new drugs. *in vitro* models are promising tools to predict drug toxicity and drug screening tests in pharmaceutical industry. In liver, isolated primary human hepatocytes (PHH) are the gold standard for drug screening approaches which representing the metabolizing profile like in vivo environments. However, these cells lose their functional metabolic features 72 hours after cultivation. In recent years some attempts have been done to extend the maintenance and functionality of PHH, including 3D cultures, micro-fluid flow cultures, 3D printing, and using synthetic and natural materials. Supportive cells in 3D culture are reliable way to improve maintanance of PHH through increase cell-cell interaction and growth factors secretion. In this work we used mesenchymal stromal cells (MSCs), human umbilical vein endothelial cells (HUVECs) as supportive cells and liver matrix derived microparticles (MPs) as natural extra cellular matrix (ECM).
Materials and Methods: Three cell types including PHH, MSCs, and HUVECs co-cultured in AgreewellTM plate and organoids harvested the day after. The MPs were fabricated with a water in oil method. The organoids were cultured in Aggrewell plate in 2 groups with and without MPs.
Results: MPs size distribution measured, that was approximately between 8-12 um. The MPs were able to incorporation into organoids successfully. The ratio of incorporation was >80%. The toxicity assay examined, data was shown that the MPs have no toxicity for cells. The ratio of MPs to cells set up in 1:2 ratio.
Conclusion: Organoids are reliable tool to investigation of drug screening and toxicity assays in liver.
Keywords: Drug Screening, Microparticles, Organoid, Hepatocyte

Ps-45: Endothelial-Derived Exteracellular Vesicles Promote *In Vitro* Human Embryonic Stem Cell-Derived Retinal Pigment Epithelium Proliferation

Hosseini Mazinani FS[1, 2*], Sanati MH[1], Baharvand H[3], Satarian L[2]
1. Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Department of Brain and Cognitive Science, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: Lsatarian@Royaninstitute.org

Background: Human embryonic stem cell-derived retinal pigmented epithelium (hESC-RPE) transplantation inspires some hopes for the treatment of retinal degenerative diseases. There are some studies that confirmed the powerful role of endothelial cells conditioned medium (ECs-CM) on RPE cells expansion. A current study aimed to investigate the effect of ECs extracellular vesicles (EVs), as an important part of paracrine factors on hESC-RPE Proliferation.
Materials and Methods: The EVs were isolated from HUVECs conditioned, then characterized by BCA protein assay, Dynamic light scattering, and western blot. 24 h post 50,000 cells/cm^2 hESC-RPE cells seeding, the 75 µg/ml EVs were added every two each day. At 9th days, both groups including PBS and EVs were considered for further experiments. To evaluate the effect of EVs on hESC-RPE proliferation, we conducted Ki-67 immunostaining and evaluate the monolayer organization by ZO1 staining.
Results: The isolated EVs passed all criteria, the mean particle diameter was 88.6 nm, and the expression of EVs marker was approved by CD81 western blot. At the 9th day, Ki-67 positive cells in EVs treated hESC-RPE were 143.41 ± 9.72 which is significantly higher compared to the control group 41.00 ± 10.39 ($P<0.0001$). The result of ZO1 staining also indicates that EVs could accelerate the hESC-RPE monolayer formation.
Conclusion: These results confirmed that the EVs secreted by HUVECs promoted expansion and monolayer formation that could be beneficial for obtaining higher numbers of hESC-RPE cells. Also, it is plausible that EVs could potentially stimulate RPE proliferation and regeneration at the early stages of retinal degenerative diseases. Further investigations on the effects of HUVECs- EVs are needed.
Keywords: hESC-RPE, HUVECs, Extracellular Vesicles, Proliferation

Ps-46: The Effect of Extracellular Vesicles Derived from Chondrocyte and Mesenchymal Stem Cells on Chondrogenesis; An *In Vitro* Study

Hosseinzadeh M*, Hosseini S, Baghaban Eslaminejad MR

Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: eslami@royaninstitute.org

Background: Osteoarthritis (OA) is the most prevalent joint disease worldwide. Given the absence of vasculature and progenitor cells, cartilage is unable to self-repair. Recently, extracellular vesicles (EV) have been suggested as a promising therapeutic approach to treat OA. However, it is necessary to identify an appropriate cellular source for isolation of extracellular vesicles with chondrogenic ability in order to promote cartilage regeneration.
Materials and Methods: Hence, this study is aimed to evaluate chondrogenic potential of EV derived from different cellular sources under *in vitro* condition. We isolated EV from rabbit chondrocyte (Cho), and bone marrow mesenchymal stem cells (rBMSCs) using a consistent centrifugation protocol. Isolated EVs from all groups were characterized in terms of size, morphology and surface markers by dynamic light scattering (DLS), scanning electron microscope (SEM) and western blotting, respectively. The isolated EV were added into micromasses composed of rBMSCs and allowed to differentiate for 21 days. Quantitative real time-PCR (qRT-PCR) and histological analysis were subsequently performed to assess the quality of chondrogenic differentiation among experimental groups.
Results: The size of isolated EVs from Cho, and rBMSCs was 151.4 ± 498.4, and 163.3 ± 1.7 nm, respectively. Western blot analysis confirmed the expression of specific markers including CD9 and CD81 in isolated EVs while annexin was not detected. The qRT-PCR results showed up-regulated expression level of Col II as a major chondrogenic marker in a group treated by rBMSCs compared to control and Cho groups. In contrary, the expression level of ColX decreased in both group. Safranin O and toluidine blue staining were positive for the group, which received rBMSCs –EVs, confirming deposition of proteoglycan.
Conclusion: Based on the results of the current study, we concluded that EVs isolated from MSCs would efficiently improve chondrogenic differentiation of rBMSCs and accelerate cartilage regeneration.
Keywords: Extracellular Vesicles, Chondrogenesis, Bone Marrow Mesenchymal Stem Cell, Chondrocyte

Ps-47: Optimization of Gp96 Upregulation in Mamospheres Regarding Cancer Vaccine Improvement

Izadpanah AH[1*], Delirezh N[1], Mahmodlou R[2]

1. Department of Cellular and Molecular Biotechnology, Institute of Biotechnology, Urmia, Iran
2. Department of Surgery, Imam Khomeini Hospital, Urmia, Iran
Email: amirhossein.izadpanah@yahoo.com

Background: Cancer stem cells (CSCs) are considered the main etiology of tumor relapses. So induction of immunogenicity against CSCs can improve cancer vaccine efficiency. CSCs can be enriched *in vitro* by sphere formation from neoplastic tissue. Heat shock proteins (HSPs) are shown to have strong antigenicity. Gp96 is one of the most known members of HSPs which facilitate cross presentation. In this project, we aimed to find optimal temperature for gp96 upregulation in spheres derived from tumor sample from a patient with grade 3 breast cancer.
Materials and Methods: Tumor sample were digested by collagenase III, hyaluronidase, and DNase. Sphere formation was done by ultra-low attachment culture condition in DMEM-F12 medium supplemented with EGF, bFGF, B27, and BSA for 22 days. Percentage of breast cancer stem cells (CD24-CD44+) was measured by Flowcytometry in D1 of culture and in secondary Mamospheres. Stemness genes expression including: Oct4, Nanog, and Sox2 was evaluated in Mamospheres by Real-time PCR. To find optimal temperature of gp96 upregulation, Mamospheres are incubated for 1 hour in two different temperatures (42°C and 43°C) then gp96 translation was evaluated by Western blot.
Results: Mamospheres were obtained after 7 days of culture and also secondary spheres formed after passaging. Flowcytometry showed, cells with CD24-CD44+ phenotype were enriched in culture period (from 3.08% in D1 to 25.98% in D22). Real-time PCR indicated Oct4, Nanog, and Sox2 expression were increased 3.8 ± 0.6, 17.8 ± 0.6, and 7.7 ± 0.8 fold respectively in comparison with MCF-7 cell line. Western blot assessment showed gp96 upregulated significantly by 1h incubation of Mamospheres in both 42 and 43°C in comparison with not heated spheres. Although band thickness of 42oc was more than 43oc, but this difference was not significant.
Conclusion: We achieved to obtain CSCs enriched Mamospheres from patient tumor tissue, and we showed with 1h incu-

bation in a temperature above 42°C gp96 would be upregulated. Gp96 upregulated Mamospheres can be used to generate more efficient cancer vaccines.

Keywords: Cancer Stem Cells, Cancer Vaccine, Heat Shock Proteins, Breast Cancer, Mamospheres

Ps-48: Treatment with Mesenchymal Stromal Cells Improve Pathological Abnormalities in ARDS

Jabbari Fakhr M[1], Mokhber Dezfouli MR[1, 2], Abbasi J[3], Shaban Malekshah D[4]

1. Institute of Biomedical Research, University of Tehran, Tehran, Iran
2. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Email: mjabbarifakhr@gmail.com

Background: ARDS can occur either direct (pneumonia, aspiration, contusion) or indirect (sepsis, trauma, pancreatitis) lung injuries, but sepsis result in the majority of cases. The pathogenesis of ARDS is complex with loss of the alveolar-capillary barrier and flooding of the airspaces with protein-rich fluid; injury to the alveolar epithelium; an invasion of neutrophils and macrophages; and fibrin deposition. The complex pathogenesis of ARDS makes animal models a necessity in the study of this disorder. MSCs exert their beneficial effects by the release of paracrine factors, microvesicles, and transfer of mitochondria, all of which have pro-inflammatory and anti-inflammatory effects on injured lung endothelium and alveolar epithelium, including enhancing the resolution of pulmonary edema by upregulating alveolar fluid clearance. MSCs also have antimicrobial effects mediated by the release of antimicrobial factors and by up-regulating monocyte/macrophage phagocytosis.
Materials and Methods: Large animal model of ARDS was induced with a single intratracheal dose of lipopolysaccharide in 10 males Shall sheep. Then, in the treatment group, BM-MSCs were isolated and cultured and 5×10^7 cells were autographed and PBS was injected in the control group intratracheally. The sheep were sacrificed 7 days after transplantation. Then, the thoracic cavity was cut, and the lungs were ligatured, dissected and removed from the chest. First, the lungs were macroscopically examined, and abnormalities were recorded. Then the tissue sections of the lungs were fixed in neutral-buffered formalin of 10%, the process was routinely done, stained with H&E and seen by Nikon Optical Microscope. Finally, the images were processed using AxioVision software, version 4.8.
Results: Sections of the lung shown severe histopathological patterns in the control group compared to the treatment group as hemorrhage in parenchyma and alveoli, severe vascular hyperemia and interstitial pneumonia, severe alveolar edema, inflammation and fibrin deposition, presence of hyaline membranes and intra-alveolar fibrin polymerization, polymorph nuclear leukocyte margination and sequestration in the capillary vessels, abundant presence of inflammatory cells, epithelial cells and other cell debris in interstitial spaces and alveoli and septal thickening inter-alveolar. But in the group recipients of BM-MSCs reduced the infiltration rate of inflammatory cells in intra-alveolar, hyperemia, hemorrhage, and edema or fibrin, and lungs structure were normal approximately and only slightly amount of thickness of the alveolar septum and edema were seen.
Conclusion: According to our clinical results, MSCs therapy could improve most of the pathological changes in this model of ADRS. In our opinion, MSC-based therapies have strong preclinical data demonstrating efficacy and can be scaled up for clinical use.

Keywords: Mesenchymal Stromal Cells, ARDS, Sheep, Pathology, Lung

Ps-49: Enhanced Osteogenic Differentiation of Mesenchymal Stem Cells Using Electromagnetic Field and Platelet Rich Plasma Modified Scaffolds

Kazem Arki M[1*], Kashef-Saberi MS[1], Hanaee-Ahvaz H[2], Kabiri M[3], Hosseinpoor H[2], Hayati Roodbari N[1], Parivar K[1]

1. Department of Biology, Islamic Azad university- Science and Research Branch, Tehran, Iran
2. Department of Tissue Engineering, Stem Cell Technology Research Center, Tehran, Iran
3. Department of Tissue Engineering, Tehran University, Tehran, Iran
Email: mandana.arki@gmail.com

Background: Recent developments in bone tissue engineering have heightened the need for development of safer and more rapid techniques along with cost-effective strategies. Utilization of autologous products has become additionally desirable and is increasingly growing. Recently, activated platelet rich plasma (PRP) has been widely used in the field of bone tissue engineering owing to its huge number of growth factors involved in osteogenesis and bone regeneration. Electromagnetic field (EMF) has proven to have inductive effects on some determination pathways of stem cells. Based on studies, biophysical and biochemicals stimuli can facilitate cell differentiation. In the present study, the effect of EMF, as a biophysical factor, and PRP, as a biochemical factor, alone and in combination with each other on osteogenic differentiation has been investigated.
Materials and Methods: To accomplish this, we coated Polyethersulfone/ Polyvinyl alcohol (PES/PVA) nanofibrous scaffolds with PRP and, cultured adipose derived stem cells (AD-SCs) on the scaffolds with electromagnetic field exposure, for 14 days. Then, common osteogenic markers were assayed by Real Time PCR. Alkaline phosphate (ALP) activity, calcium mineral deposition and Alizarin red staining were performed.
Results: The results revealed that the best osteogenic differentiation occurred when cells were cultured on PRP coated PES/PVA scaffold. The exposure of electromagnetic field and presence of osteogenic factors did not show synergistic or additive effects. Among various combinations of already approved osteo-inductive factors, namely PRP, EMF and exogenic osteogenic factors, the best result was achieved by the simplest and least cost strategy, i.e. merely by PRP coating of the surface.
Conclusion: PRP modified PES/PVA scaffolds by itself can maximally induce osteogenesis without the need for any external physical and/or biochemical stimulations. The major contribution of this paper to the current research on bone regeneration is to establishing the effects of PRP-coated scaffold on osteogenesis.

Keywords: Bone Tissue Engineering, Adipose Derived Mesenchymal Stem Cell, Electromagnetic Field, PRP, Nanofibrous Scaffold

Ps-50: Lipid Desaturation in Normal Human Ovarian Surface Epithelium Cell Cultures

Keshavarz Sh[1, 2*], Moshtaghioun SM[1], Eftekhar M[3], Farashahi Yazd E[2, 4], Mirmohammadi R[5], Mohammadi M[3], Akyash F[2, 3], Aflatoonian B[2, 4, 6]

1. Department of Biology, Faculty of Science, Yazd University, Yazd, Iran
2. Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
4. Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
5. Department of Obstetrics and Gynecology, Faculty of Medical Sciences, Islamic Azad University, Yazd Branch, Yazd, Iran
6. Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: moshtaghiun@yazd.ac.ir, b.aflatoonian@ssu.ac.ir

Background: Latest investigations on ovarian germ-line stem cells (GSCs) have changed the idea of the existence of the fixed number of oocytes following the birth of the mammalian females. The origin of ovarian GSCs are claimed to be from the epithelium surface. These ovarian surface epithelium (OSE) cells display self-renewal capacity and identified by both specific stem cells and germ cell markers such as SSEA4 and VASA. Recently, we have assessed the stemness of the OSE isolated and cultured from normal human ovaries. In the current study, it is shown that, following the expansion, cells in the culture display lipid desaturation which is known as a metabolic marker of ovarian cancer stem cells (CSCs).
Materials and Methods: The biopsies of the OSE were taken by scrapping from the patients undergoing operation; following obtained written informed consent from patients. Samples were transferred to the lab and human OSE cells were isolated and cultured after rinsing and centrifugation. The proliferating cells were undergone immunoflorescent (IF) staining with SSEA4 and VASA antibodies. Q-PCR was performed to assess the level of the expression of the VASA in different passage numbers. The level of the progesterone was detected using ELISA.
Results: OSE derived cells from normal human ovaries expressed both VASA and SSEA4 while proliferating in early passages. However the level of the VASA expression was down regulated later as revealed by Q-PCR. Interestingly, cells in later cultures showed lipid desaturation which is known as metabolic markers of human ovarian cancer stem cells in recent publications. ELISA data showed no sign of progesterone secretion by the cells in their initial cultures.
Conclusion: Our data confirms others reports for existence of stem cells from human OSE. Moreover, some of the cells in later cultures showed lipid desaturation. Recent articles reported that lipid desaturation is a metabolic marker of human ovarian CSCs. This finding indicates that ovarian CSCs and GSCs may have similar origin which is OSE.
Keywords: Germ-Line Stem Cells, Lipid Desaturation, Ovarian Cancer Stem Cells, Ovarian Surface Epithelium Cells

Ps-51: Development of An Automatic Decellularization Device of Biological Tissues and Rat Renal Tissue Decellularizing with Sodium Dodecyl Sulfate

Keshvari MA[1*], Mahmudpour M[1], Daneshi S[2], Miri MR[1], Azari H[1], Mirzae F[1], Nabipour I[1], Tamadon A[1]

1. The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University Of Medical Sciences, Bushehr, Iran
2. Department of Anatomical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
Email: amintamaddon@yahoo.com

Background: End stage renal disease (ESRD) caused by chronic diseases such as diabetes and hypertension is a rapidly-growing reason for morbidity and mortality worldwide. ESRD usually leads to either dialysis or renal transplantation which is costly and also hardly available for majority of people. To surpass the current limitations, such as shortage of donor and unavailability, using tissue engineering as a new hope for people with ESRD is considered. The first step is the fabrication of an intact decellularized kidney scaffold.
Materials and Methods: We developed an automatic decellularization device to perfuse and decellularize male Wistar rats' kidney with sodium dodecyl sulfate (SDS). After anesthesia with ketamine and xylazine, a longitudinal abdominal incision was made and the left kidney, aorta, vena cava and ureter were identified. Then heparin was injected intravenously to prevent blood clotting. Renal artery was cannulated and perfused with phosphate buffer saline (PBS) for 2 hours at a flow rate of 2 ml/min. To remove blood from kidney, renal vein was cut. Then, kidney was arterially perfused with a 1% SDS solution for 4 hours at a flow rate of 1 ml/min and then another 2 hours with PBS to remove the remaining detergent in scaffold. Physiological pressure was maintained within 62 to 107 mmHg (it is assumed to be rat kidney pressure) by setting the device flow rate between 1 to 2 ml/min through the entire duration of the decellularization process. The decellularized scaffold was stained with hematoxylin and eosin, Verhoeff's Van Gieson, Masson's trichrome, and Alcian blue to check cell removal and examine elastin, collagen and Glycosaminoglycans (GAGs), respectively.
Results: We developed an automatic and digital decellularization device for decellularizing the biological tissue. Our data showed complete cell removal and effectively preservation of extracellular matrix (ECM) architecture in rat kidney.
Conclusion: SDS is a promising detergent that could be a useful decellularization agent to produce acellular kidney scaffold much alike native kidney scaffold. An automatic and digital decellularization device could facilitate kidney decellularization.
Keywords: Automatic Decellularization Device, Kidney, Rat, Sodium Dodecyl Sulfate

Ps-52: Optimized Method for Manufacturing and Purifying Nkx2.2 Modified-Mrna for Therapeutic Applications

Khayamabed R[1, 2*], Rezaei N[3], Dormiani K[3], Homayouni Moghadam F[1], Nasr Esfahani MH[1]

1. Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Biology, ACECR Institute of Higher Education, Isfahan, Iran
3. Department of Molecular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: f_homayounim@yahoo.com

Background: Nkx2.2 is a homeodomain-containing transcrip-

tion factor, robustly expressed in pancreatic islets and central nervous system (CNS). It is required for final differentiation and maturation of pancreatic β-cells to produce insulin and oligodendrocyte precursor cells to remyelinate demyelinating lesions. The necessity of Nkx2.2 overexpression with the aim of clinical applications, needs an efficient and safe system to produce this factor. Synthetic modified-mRNAs have considered as an effective method for delivering genetic information and inducing transient expression without genetic modification. Modified nucleotides reduce the immunogenicity of therapeutic RNAs likewise increase their stability. The goal of this study was to optimize the best condition for producing large amounts of Nkx2.2 modified-mRNAs with less immunogenicity.
Materials and Methods: Linearized pMRNA-Nkx2.2 and T7-Nkx2.2-PolyA PCR product were served as template for *in vitro* Transcription (IVT) using NEB HiScribeT7-Kit. Transcribed mRNAs were purified by phenol-chloroform, LiCl and Qiagen-RNeasy column. To analyze the mRNA concentration, purity and integrity, spectrophotometer as well as agarose gel were used.
Results: Our result demonstrated that the yield of produced modified-mRNAs was the same in two different template conditions; equal number of molecules and equal concentration. However, the amount of unintended double-stranded RNAs (dsRNAs) was lower using linearized vector. Additionally, all aforementioned characteristics of transcribed modified-mRNAs was significantly higher in LiCl purification method. Moreover, increased centrifugation time, enhanced the purification efficiency.
Conclusion: In conclusion, using linearized vector as IVT template produces less dsRNA, which ultimately resulting in less immune system stimulation. Furthermore, LiCl method with higher centrifugation time is the best condition for purifying modified-mRNAs.
Keywords: Nkx2.2, Pancreatic Islets, Central Nervous System, Modified-Mrna, *in vitro* Transcription

Ps-53: Cytotoxic Property of Trifolium Pretens L. Hydroalcoholic Extract On Prostate Cells

Khazaei M[*], Pazhouhi M, Khazaei F, Salimi E

Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: mkhazaei1345@yahoo.com

Background: Background: Prostate cancer is one of the most common cancers and the second leading cause of cancer-related death among men. It accounts for 33% of all newly diagnosed malignancies, and usually develops over 50 years of age. Trifolium pratense L. has been suggested for cancer treatment in traditional medicine. In this study the effect of T. pratense hydroalcoholic extract on two prostate cell lines and normal fibroblast cells was investigated.
Materials and Methods: LNcap and PC3 cell lines were seeded in cell culture flasks containing Roswell Park Memorial Institute 1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum (FBS) without antibiotics. The medium of normal fibroblast cells was DMEM/F12. The cells were treated with 12.5, 25, 50, 100, 200, 400 and 800 µg/mL of T. pratense extract for 24, 48, 72 and 96 hours. Cell viability was evaluated using trypan blue staining, MTT assay, and lactate dehydrogenase activity measurement. Nitric oxide production was measured using Griess reaction.
Results: All the cell lines responded to the cytotoxic effect of the plant extract in a dose- and time-dependent manner. The LNCap cancer cells, however, were more sensitive to the extract as shown by its IC50 values for 24, 48, and 72 hours. Moreover, the extract exhibited selective cytotoxicity in normal fibroblast cells with higher IC50 values. The LDH activity in cell culture medium increased with increasing concentration of the extract. So, cytotoxicity of extract is accomplished with plasma membrane damage.
Conclusion: T. pratense showed an anti-cancer property in prostate cancer cell lines.
Keywords: Prostate Cancer, Cell Culture, Trifolium Pretense L

Ps-54: Induction of Autophagy Cell Death in The Human Prostate Cancer Cell Lines by Trifolium Pratens Hydroalcoholic Extract

Khazaei F[*], Pazhouhi M, Khazaei M

Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: fatimakhazaei7@yahoo.com

Background: Autophagy has been considered as a cell death mechanism. Most of the cancer cells have defect apoptosis pathway and autophagy could function as a backup for cell death. Prostate cancer is one of the most common cancers and the second leading cause of cancer-related death. Studies have suggested that consumption of a plant-based diet have a useful effect on the cancer therapy. Trifolium pratense has been suggested for cancer treatment in traditional medicine book. The aim of the present study was to determine the effect of T. pratense hydroalcoholic extract on two human prostate cancer cell lines and normal fibroblast cells.
Materials and Methods: LNCap and PC3 cell lines were seeded in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) without antibiotics. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO^2. The cells were treated with 12.5, 25, 50, 100, 200, 400 and 800 µg/mL of T. pratense extract. To analyze the autophagy induction, AVOs, which consist predominantly of autophagosomes, and autolysosomes, were quantified using AO staining. The effects of various concentration of T. pratense extract on the expression level of some autophagic-related genes were analyzed by real-time PCR.
Results: T. pratense extract induced cell death through the activation of autophagy. The extract induced autophagy in a concentration-dependent manner. The LNCap cell line was a bit sensitive to the extract when compared with other cell line.
Conclusion: T. pratense showed an anti-cancer property via induction of autophagy cell death in prostate cancer cell lines.
Keywords: Autophagy, Prostate Cancer, Trifolium Pretense L

Ps-55: Effects of Metformin on Viability of Rat Epidermal Neural Crest Stem Cells: An *In Vitro* Study

Khodabakhsh P[1*], Dargahi L[2], Givzad N[3]

1. Department of Pharmacology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Physiology, University of Saskatchewan, Saskatchewan, Canada
Email: p.khodabakhsh@sbmu.ac.ir

Background: Epidermal neural crest stem cells (EPI-NCSCs) are multipotent stem cells able to undergo self-renewal and to generate all major neural crest derivatives, including neurons, Schwann cells, smooth muscle cells, bone/cartilage cells, and pigment cells. Previous studies reveal that insulin in neurosphere cultures promotes proliferation and differentiation of neural progenitors. These observations are highly relevant as the field of regenerative medicine moves closer to the development of tools to implement stem cell therapy for replacing damaged or lost neurons as a treatment for neurodegenerative diseases. Thus, the insulin signaling pathway may provide a rational strategy to more efficiently proliferate *in vitro* and/or to improve the differentiation efficiency.
Materials and Methods: Here, we studied the effects of metformin (0.1, 1, 10, 100, 1000 and 10000 nM) on viability of EPI-NCSCs isolated from bulge of rat hair follicles through MTT assay.
Results: The cells treated with most of the doses of metformin (0.1-100 nM) increased cell viability, dose dependently. It was also shown that cell viability was reduced in a decreasing manner in doses more than 1000 nM.
Conclusion: Thus, metformin represents an optimal candidate neuro-regenerative agent that is capable of expanding the adult EPI-NCSC population. This study represents a vital step forward in the optimization of human EPI-NCSCs-based transplantation to promote neural damage recovery.
Keywords: Insulin, Metformin, Epidermal Neural Crest Stem Cells, MTT, Rat

Ps-56: A Systems Approach Identifies The Holistic Map of Diabetic Nephropathy

Kiyanpour F, Abedi M, Gheisari Y

Isfahan University of Medical Sciences, Regenerative Medicine Research Center, Isfahan, Iran
Email: ygheisari@gmail.com

Background: Chronic diseases are the main cause of death and disability in human society. Despite different investigations, the molecular mechanisms are not fully discovered. Systems biology approaches can enhance our knowledge about these complex diseases. Diabetic nephropathy (DN), as a chronic disease, is one of the major complications of diabetes mellitus patients. In spite of huge investigations, a holistic view of this complex disorder has rarely been done. In this study, we have reanalyzed a microarray dataset of the kidney cortex of diabetes mice, to identify key genes and functions in this complex condition.
Materials and Methods: A microarray datasets GSE86300 (kidney cortex) which is the expression profile of db/db mice and healthy individuals were downloaded from Gene Expression Omnibus (GEO) database. The quality of each dataset was evaluated by unsupervised hieratical clustering and princaple component analysis (PCA) by GGplot2 package of R software. Using GEO2R and considering adjusted P-value<0.05, differentially expressed (DE) genes were identified. Next, transcription factors (TF) regulating DE genes and kinases controlling TFs by with P-value≤0.05 harvested using EnrichR database. After that, using CluePedia application of Cytoscape software version 3.5.1, the multilayer networks of DE genes, TFs, and kinase were constructed. NetworkAnalyser tool of Cytoscape was used for network analyses. In continue, pathways and gene ontology (GO) terms were collected using ClueGo application of Cytoscape.
Results: The quality of dataset was acceptable as PCA separated disease and normal samples. 1042 DE genes, 35 TFs, and 41 kinases was harvested, in this dataset. Among central DE genes, that revealed by network's topology analysis, Wt1, Mapk family, and Sox2 was observed which have been previously confirmed in the pathogenesis of DN. Also, some new genes like Suz12 and Rxra were introduced for the first time. Pathway enrichment analysis identified some well-known role player in DN such as FoxO and ErbB signalling pathway, and likewise MAPK signalling cascade. Enriching Neurotrophin signalling pathway was in line with our previous unpublished data on DN and validated in this one. In addition, small molecule metabolism, lipid metabolic process, and anion binding were among GO-term enrichment analysis.
Conclusion: In conclusion, here we have shown the central genes, key signalling pathways and GO-terms in DN. These findings deepen our knowledge about the pathogenesis of this complex disease.
Keywords: Diabetic Nephropathy, Differential Expressed Gene, Microarray Analysis, Pathway Enrichment Analysis

Ps-57: Neural Stem Cell Derived Extracellular Vesicles Prevents Microgliosis in Rat after Permanent Focal Ischemia

Mahdavipour M[1], Seifali E[1], Hassanzadeh GH[1], Shekari F[2], Aligholi H[3], Abkari M[1]

1. Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Neuroscience, School of Advanced Medical Science and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
Email: akbarimo@tums.ac.ir

Background: Ischemic stroke is one of the most common causes of death and neurological disability worldwide. Neural stem cells (NSCs) have been suggested to reduce neuroinflammation, in part through their secretion factors. Extracellular vesicles (EVs) are membrane-bound vesicles suggested to be potential exogenous therapeutic tools. Here, we studied whether NSC derived EVs (NSC-EVs) have anti-inflammatory effects on microglia and prevent microgliosis.
Materials and Methods: EVs were isolated from neural stem cell culture by ultracentrifugation and characterized by enriched protein expression, size, and morphology. Middle cerebral occlusion artery (MCAO) used as rat ischemic stroke model. Seven days after stroke, the modified neurological severity score (mNSS) test was performed to measure functional recovery. Microglial marker Iba1 immunoreactivity was evaluated in brain coronal sections by immunohistochemistry technique in sham-injury, ischemia and EV treated groups.
Results: Animals (n=3) treated with NSC-EVs significantly decreased Iba1 immunoreactivity to the baseline state. Also, displayed consistently better neurological function with mNSS score reduction.
Conclusion: NSC-EVs administration prevents microgliosis in the ischemic brain and improves functional recovery.
Keywords: MCAO, Neural Stem Cell, Extracellular Vesicle, Microglia, Mnss

Ps-58: Cancer Stem Cell Enrichment and Neurosphere Formation in Isolated Cells from Glioblastoma Tumor Sample

Maleki Sh*, Delirezh N, Zand S

Department of Pathobiology, Faculty of Veterinary Medicine, Urmia, Iran
Email: n.delirezh@urmia.ac.ir

Background: Glioblastoma multiform (GBM) is the most common malignancy in the central nervous system accounting for 45% of the malignant brain tumor and the most lethal type of brain cancer. The major problem on the way of effective treatment for this type of cancer is presence of group of cells that called cancer stem cell in tumor mass. Research has shown that cancer stem cell paly role in metastasis, tumor resistance to chemotherapy and radiotherapy and tumor recurrence after treatment.
Materials and Methods: At first glioblastoma cells were isolated from tumor tissue then a group of cells were cultured in NMGF (Neurosphere medium supplemented with Growth Factors) and another one were examined for cancer stem cell population (CD133+) by flow cytometry. After 25 days, a group of cells were checked for CD133+ by flowcytomery.
Results: After 7 days first neurosphere formed and Result of CD133+ flow cytometry in zero day was 1.58% and after 25 days was 5.64%.
Conclusion: Neurosphere formation shows increasing of cancer stem cell population and Result of CD133+ flow cytometry indicate the cancer stem cell present that was 1.58% in zero day, was increased to 5.64% in 25 day. Sphere formation in NMGF was supported by result of CD133 flow cytometry in 25th day.
Keywords: Glioblastoma, Brain Cancer, Cancer Stem Cell

Ps-59: Evaluation of Invasion and Migration Ability of HCC Model (Huh-7, SK-Hep-1, Hep-3B) after Treatment with A Natural Ligand of HNF4α

Miri Lavasani Z[1], Zahmatkesh E[1], Farzaneh Z[1], Haeri A[2], Piryaei A[3], Vosough M[1, 4]

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 Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Pharmaceutics, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Cell Biology & Molecular Genetics Science, University of Science and Culture, ACECR, Tehran, Iran
Email: masvos@yahoo.com

Background: Nowadays, considering the growing number of liver cancer and failure in aggressive treatments differentiation therapy could be considered as a promising strategy for recurrence inhibition. Numerous efforts have been made to promote differentiation in dedifferentiated hepatocarcinoma cells. Accordingly, HNF4a as a curtail transcription factor in hepatocytes, emerge to be a kay player in differentiation induction by mediating EMT-MET process. Enhance of HNF4a expression appeared to decrease invasion in carcinoma cells by suppressing Snail and decreasing EMT markers. The current study aims to address invasiveness in hepatocarcinoma cells after treatment by a natural ligand.
Materials and Methods: We obtained 3 hepatocellular carcinoma (HCC) cell lines form Royan cell bank. Sk-Hep-1 is selected for the study. Then, these cells treated by CLA and BIM5078, as an antagonist of HNF4a, as a negative control. The effects of CLA on cellular behavior were measured by assessing cell viability and proliferation rate at different time points. The expression level of HNF4a, invasiveness and EMT marker genes were assessed using quantitative real time polymerase change reaction (qRT-PCR). Moreover, migration and colony formation ability were assessed by scratch and colony formation assay.
Results: MTT results showed that CLA and BIM5078 and their vehicles at applied concentration had no cytotoxic effect on cell survival rate. “Orangu test” results demonstrated that CLA significantly decreased the cell proliferation rate, whereas BIM5078 increased it. Quantitative RT-PCR results showed that CLA enhances the expression of HNF4a and decrease EMT marker genes. Furthermore, migration and colony formation ability of cancerous cells significantly decreased after treatment with CLA.
Conclusion: Our data revealed that CLA can induce well-differentiation in hepatocarcinoma cells particularly on its migration ability by HNF4a induction.
Keywords: Hepatocellular Carcinoma, Differentiation Therapy, Epithelial Mesenchymal Transition, HNF4α, Snail

Ps-60: Resveratrol Effect on Viability and Nitric Oxide Secration in Human Breast Cancer Cell Lines

Mirzapur P*, Khazaei MR, Khazaei M

Fertility and Infertility Research Center, Kermanshah Uninversity of Medical Sciences, Kermanshah, Iran
Email: mkhazaei1345@yahoo.com

Background: Breast cancer is the most common cancer of women. Numerous therapies have been identified and used for this cancer. Natural compounds have been used as potential cancer chemopreventive agents. Resveratrol is a natural polyphenolic amzing compound. The aim of present study was to investigate the effect of resveratrol (Res) on viability of breast cancer cell lines (MCF7 and MDA-MB-231) and nitric oxide changes.
Materials and Methods: MCF7 and MDA-MB-231 Cells were treated with Res (20μM) during 72 h period. Cell viability (MTT assay), and nitric oxide (NO) production (Griess method) were evluated.
Results: Viability of MCF7 and MDA-MB-231 cells were decreased significantly (P=0.000) by Res, After 72 hours and it was 84.84 ± 2.1 %. Nitric oxide production by MCF7 and MDA-MB-231 cells exposed upon Res showed a significant reduction (P=0.000). NO production from MCF7 and MDA-MB-231cells decreased in a time-dependent manner. It was 22.57 ± 3.2 μM in MCF7 medium (P=0.001), and 18.41 ± 1.4 μM in MDA-MB-231cell medium (P=0.000).
Conclusion: Res decreased NO production in a time-dependent manner and its anti-tumor effect appears to be created through angiogenesis inhibition or induction of cell cycle arrest and apoptosis in cancer cells.
Keywords: Breast cancer, Resveratrol, Nitric Oxide, Viability

Ps-61: Combination Effect of ATO/THAL On PI3K/AKT/Mtor Pathway in AML Cell Lines

Mohammadi Kian M[1, 2*], Nikbakht M[1, 2], Haghi A[1, 3], Salemi M[1,

[2], **Mohammadi S[1, 2]**

1. Hematology Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
2. Hematologic Malignancies Research Center, Tehran University of Medical Sciences, Tehran, Iran
3. Young Researchers and Elite Club, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
Email: mahnazmohammadikian@gmail.com

Background: Acute myeloid leukemia (AML) is a heterogeneous group of malignant that is caused by an uncontrolled protein of clonal neoplastic cells and its accumulation in the bone marrow.AML resistant cell line (CD34+) is 10-15 times more susceptible than sensitive cell line (CD34-) to chemotherapy Anticancer effect. PI3K/AKT/mTOR signaling cascade is crucial to many widely different physiological processes such as cell survival and apoptosis. The previous studies demonstrated the cytotoxic activity of Arsenic trioxide (ATO) in many cancers. Thalidomide (THAL) is also an angiogenesis inhibitor it blocks the survival in cells. Therefore, investigating the effect of ATO and THAL on PI3K/AKT/mTOR and PTEN mRNA expression is the primary goal of this study.
Materials and Methods: KG-1 and U937 cells (respectively as resistant and sensitive cell lines to chemotherapy) treated with ATO and THAL with different dose and time manner. Cell proliferation was evaluated by MTT assay. The rate of apoptosis was measured by flow cytometry (Annexin-V/PI) also cell cycle analysis was done by flow cytometry (PI). Furthermore, the effect of cited compounds on the mRNA expression level of PI3K/AKT/mTOR and PTEN were measured by Real Time-PCR.
Results: Effective dose and IC50 of THAL in KG-1 and U937 respectively were 80µM and 60µM. Effective dose and IC50 of ATO in KG-1 and U937 respectively were 1.618µM and 1µM. Annexin-V/PI staining indicated that ATO and THAL can induce apoptosis in both cell lines. Cell cycle analysis showed that cells arrested in subG1/G1 in the presence of ATO/ THAL. Results of Real Time-PCR showed that the level of mRNA Expression of PI3K/AKT/mTOR was decreased and PTEN was increased in both cell lines.
Conclusion: According to the obtained results combination of THAL and ATO has an anti-leukemic effect by downregulating of PI3K/AKT/mTOR and upregulating of PTEN.
Keywords: AML, Arsenic Trioxide, Thalidomide, PI3K

Ps-62: An Injectable Hydrogel of Kappa-Carrageenan- Dopamine Functionalized Graphene Oxide with Shear-Thinning and Mechanically Robust Characteristics for Soft Tissue Engineering

Mokhtari HR*, Tavakoli Sh, kharaziha M, Karimzadeh F

Department of Material Enginnering, Isfahan University of Technology, Isfahan, Iran
Email: mokhtarirezahamid@gmail.com

Background: Recently there is a wide range of request for tissue transplantation and lack of immune sources stimulates researchers to recreate organs from natural materials. methacrylate-Kappa-carrageenan (KaMA) has been proposed as a suitable network, which can be able to crosslinked ionically and chemically. Mechanical and physical tunable properties of crosslinked hydrogel is unique factor for crosslinked hydrogels to mimicking target soft tissue
Materials and Methods: Kappa-carrageenan was methacrylated with 4%v/v degree and was crosslinked through a chemical and physical process with UV light and KCl respectively. Moreover different percentages of dopamine functionalized graphene oxide was initially added to primary hydrogel solution.
Results: Graphene oxide-functionalized with polydopamine (PD) seems to be promising additive to structure KaMA, that can enhance shear thinning behavior of hydrogel through interaction of active catechol group belonging to PD with other moieties in structure of hydrogels. In addition we claimed that these interaction also promote mechanical properties of hydrogels after crosslinking. In our find, encapsulation of 20wt % GOPD enhanced toughness (6-folds) and compressive strength (8-folds) prominently. Moreover, with the presence of these secondary bonds, this hydrogel can be considered as a self-healable hydrogel due to recreation the broken bonds. The incorporation of GOPD significantly improved swelling ration and decrease degradation rate, while with increasing amount of GOPD cell behavior were influenced, cell attachment and spreading promoted
Conclusion: KaMA-GOPD hydrogel can be used to deliver cells and biological molecules through the injection process and presented as suitable biomaterial ink for 3 Dbioproiting.
Keywords: Shear Thinning, Self-Healing, Nanocomposite Hydrogels, Dopamine, Kappa-Carrageenan

Ps-63: PCL-PANi_GEL Nanofibrous Scaffolds Potential in Stem Cell Tissue Engineering

Moridi K[1*], KarbalaeiMahdi A[1], Jalili A[3], Salimi A[3]

1. Department of Nanotechnology, Azad University of Pharmaceutical Sciences, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Royan Stem Cell Research Center, ACECR, Tehran, Iran
3. Department of Nanotechnology, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: Kaykhosro.moridi@gmail.com

Background: The structure of biocompatible scaffolds is one of the biggest concerns of tissue engineers. Ideally, a scaffold should mimic structural and biological functions of extra-cellular matrix (ECM); both in terms of physical structure and chemical component. Scientists have found that nanofibrous scaffolds can provide a suitable environment for cell attachment, proliferation and differentiation and thus, play an important role in tissue engineering. Gelatin is a biocompatible, biodegradable and a natural protein that is derived from collagen which is a main component of ECM. Polycaprolactone is a semi-crystalline, biodegradable soluble polymer and as a result, it has stimulated a lot of research on its potential and applications in tissue engineering. Conductive polymers, due to their direct electrical stimulation capabilities, have received a great deal of attention. In this study, a variety of aligned and random PCL-PANI-GEL nanofibrous scaffolds were electrospun to determine their potential for tissue engineering regards. In addition, the osteogenic differentiation of MSCs cultured on the prepared scaffolds was investigated.
Materials and Methods: PCL-PANI-GEL nanofibrous scaffolds were prepared by Electrospinning, using a bi-electrospun method in order to achieve a feasible outcome. The morphology of nanofibrous scaffolds were observed and studied by SEM

imaging. The porosity of bimodal foams was measured using a specific gravity bottle based on Archimedes' Principle. Tensile properties were evaluated by an Instron Tensile Testing Apparatus (5566-Applied Science Co., Ithaca, NY). In order to evaluate the viability of nanofibrous scaffolds, MTT assay as well as Acridine Orange staining tests were performed. Furthermore, to fully investigate the fibers' potential for tissue engineering, osteogenic differentiation of MSCs were performed. MSCs were seeded on nanofibers and after 14 days of differentiation, Alkaline Phosphatase activity, Alizarin Red Staining and Calcium Content test were performed to evaluate the fibers' potential for stem cell differentiation.
Results: Two candidates were chosen from the 6 fabricated nanofibers. SEM imaging showed that the latter 2 were suitable for further tests. FTIR spectra of PCL-PANI-GEL nanofibers confirmed the chemical characterization of electrospun nanofibers. MTT results supported the biocompatibility of the scaffolds. Then, MSCs were cultured on the scaffolds and then, differentiated to osteoblasts. Alkaline Phosphatase activity, Alizarin Red Staining and Calcium Content test confirmed the nanofibers potential for stem cell differentiation.
Conclusion: In conclusion, we were able to reach an appropriate setup for PCL, Gel and PANI combination. Additionally, PANI-PCL-GEL electrospun fibers could be used as an appropriate scaffold for efficient regeneration of bone defects. These synthetic nanofibers show promising applications in bone tissue engineering. Various assessments such as ALP activity, calcium content, alizarin red, mineralization staining and SEM micrographs demonstrated that the random and aligned electrospun scaffolds are appropriate for MSCs osteogenesis. In the future, these scaffolds can be used in in vivo analysis which will have a positive effect on bone healing in critical-size bone defects.
Keywords: Nanofiberous Scaffolds, Stem Cell, Tissue Engineering

Ps-64: The Effects of Silk Nanofiber Electrospun Scaffold On The Proliferation of Neonatal Mouse Spermatogonial Stem Cells

Narimanpour Z[1*], Nazm Bojnordi M[1], Ebrahimi S[2], Saremi J[2], Ghasemi H[1]

1. Department of Anatomy & Cell Biology, Immunogenetic Research Center, Faculty of 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
Email: bojnordi@modares.ac.ir

Background: Nano fibrous scaffolds improve *in vitro* proliferation of stem cells via serving a similar and appropriate Extra Cellular Matrix (ECM), guiding the cell attachment. In this study the stimulatory effects of a Silk nanofibrous scaffold was evaluated on maintain and *in vitro* proliferation rate of mouse spermatogonial stem cells (SSCs).
Materials and Methods: Mouse neonatal spermatogonia stem cells were isolated from mouse testis (2-4days) using a modified two-step mechanical and enzymatic digestion. After seeding SSCs in the sterilized silk scaffolds, they were incubated in DMEM/F12 medium. The attachment potential of SSCs were evaluated using SEM at days 3 and 5th days post seeding on the scaffold. MTT assessments and DAPI staining was used for viability and attachment of seed cells. Specific spermatogonia markers includes; Stra8, DAZL and Piwil2 were investigated via real time PCR and immunocytochemistry techniques.
Results: MTT dates showed that cell viability rate of seeded SSCs on silk scaffold showed a significant increase in comparison with the control group. The viability rate of SSCs post two weeks culture was 76.90% ± 4.16% and showed a significant increase (87.40% ± 0.73%) in cultured SSCs on silk scaffold. The attachment and proliferation of SSCs was increased in the presence of Silk electrospun scaffold in compare to monolayer sells. Real time PCR dates proved an upregulation in expression of specific spermatogonial markers e.g.; Stra8, DAZL and Piwil2 in SSCs grown on silk scaffold, in compare with control group.
Conclusion: It is concluded that Silk nanofibrous scaffold improves maintain and viability of neonatal mouse spermatogonial stem cells, and stimulates the proliferation rate during cultivation. The results of our study suggests fabrication of the electrospun silk scaffold as a biological substitutes for efficient propagation of spermatogonial stem cells which is a crucial step in tissue engineering for reproductive medicine.
Keywords: Spermatogonia Stem Cells, Scaffold,Silk, Proliferation, Tissue Engineering

Ps-65: Transplantation of Human Bone Marrow-Derived Mesenchymal Stromal Cells Combined with GLP1 Agonist in Diabetic Non-Human Primates

Navabi R[1*], Hajizadeh-Saffar E[2], Hajinasrollah M[3], Jenab Y[4], Rabbani Sh[4], Baharvand H[5], Negahdari B[6]

1. Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Science, Tehran, Iran
2. Regenerative Medicine, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Stem Cells and Developmental Biology, Animal Core Facility, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran
4. Tehran Heart Center, Tehran University of Medical Science, Tehran, Iran
5. Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
6. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Science, Tehran, Iran
Email: baharvand@royaninstitute.org

Background: Type 1 diabetes (T1D) represents as an autoimmune disease that β cells selectively destroy by immune cells and patients are dependent on exogenous insulin for blood glucose regulation. At the time of clinical diagnosis of T1D, 20-30 % of β cells remain in the pancreas. Modulating immune system concurrent to promoting beta cell function is one of the goals to develop innovative therapies of T1D. So, the preservation of these remaining β cells is one of the best therapy goals to enhance the secretion of endogenous insulin. Mesenchymal stem cells (MSCs) have immunomodulatory properties. Among various drugs for T1D, GLP-1 agonists increase regeneration of β cells. So the current research project intends to investigate the effect of combination therapy of human bone marrow-derived MSCs (hBM-MSCs) transplantation along with administration of the GLP-1 agonist (liraglutide) in new-onset diabetic rhesus Macaque mulatta monkey.
Materials and Methods: We developed a rhesus monkey model of diabetes induced via multiple low dose intravenous injection of 30 mg/kg b.w. streptozotocin. 1.5×10^6 hBM-MSCs/

kg b.w. were transplanted into the celiac artery through angiography method in two injections and also 1.8 mg/day Liraglutide was administered subcutaneously up to 8 weeks to the combination group. The animals were followed by functional, immunologic and histologic assessments.
Results: Transplantation of 3×10^6 hBM-MSCs/kg b.w. combined with liraglutide daily injection, prevented hyperglycemia progression up to 10 weeks and improved body weight, hemoglobin A1c, fructosamine and beta cell function compared with the hBM-MSCs/monotherapy and the sham groups. With protective effect of this combined treatment, Foxp3 T- regulatory cells markedly increased and inflammatory cytokines of IL-6 and IL-1 Beta decreased compared with the hBM-MSCs/monotherapy and the sham groups. Also in morphological analysis of pancreatic tissue, lower inflammation and more normal islet were observed compared with the sham group.
Conclusion: Our findings demonstrate that combination therapy of hBM-MSCs+Liraglutide in rhesus monkey model of diabetes, exhibited superior immunomodulation and regeneration properties in comparison with the hBM-MSCs/monotherapy and the sham groups.
Keywords: Mesenchymal Stem Cell, Immunomodulation, GLP-1 Agonist, Regeneration, Non-Human Primate

Ps-66: Silk Nanofibrous Scaffold Improves Glial Differentiation of Embryonic Stem Like Cells

Nazm Bojnordi M[1*], Ebrahimi-Barough S[2], Vojoudi E[2], Saremi J[2]

1. Immunogenetic Research Center, Department of Anatomy & Cell Biology, Faculty of 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.
Email: bojnordi@modares.ac.ir

Background: Stem cell therapy accompanied with optimal scaffolds, is a promising treatment in neural tissue engineering strategies via serving an appropriate microenvironment to induce neuroglial differentiation in damaged neural tissue. Here we fabricated a silk Nano fibrous scaffold as a microenvironment for glial guiding differentiation of Embryonic stem like cells.
Materials and Methods: Embryonic stem like cells, were seeded and cultured on Silk scaffolds. The glial differentiation was induced using a modified technique includes culturing in the presence of Retinoic acid following addition of neurobasal medium supplemented with 10 ng/ml Epidermal Growth Factor, 20 ng/ml basic Fibroblastic Growth Factor for 10 days. The glial differentiation was analyzed via the evaluation of specific markers; Nestin, Oligodendrocyte transcription factor (Olig2) and O4 via immunocytochemistry and real-time technique.
Results: Our dates proved that silk scaffold support the differentiation of Embryonic stem like cells cells in to glial cells. The expression of Nestin, OLIG2 and O4 markers were significantly higher in the experiment group in compare to monolayer control group.
Conclusion: This study suggests fabrication of the electrospun silk Nano fibrous scaffold as a biological substitutes for glial differentiation of stem cells that is a crucial step in tissue engineering for neural tissue repair and regeneration.
Keywords: Silk, Differentiation, Tissue Engineering, Embryonic Stem Cells, Scaffold

Ps-67: Neurogenic Differentiation of Human Dental Pulp Stem Cells by Optogenetics Stimulation

Niyazi M[1*], Zibaii MI[2], Dargahi L[3], Ghasemi Hamidabadi H[1, 4], Nazm Bojnordi M[1, 4], Chavoshinezhad S[3], Heravi M[3], Hosseini M[2], Karimi Tar H[2], Sadeghi E[2]

1. Department of Anatomy and Cell Biology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
2. Laser and Plasma Research Institute, Shahid Beheshti University, Tehran, Iran
3. Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Anatomy and Cell biology, Immunogenetic Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
Email: hatefdr@gmail.com

Background: The purpose of this study is to analyze the effect of optogenetics stimulation in propagation of human dental pulp stem cells (hDPSCs) and neurogenic induction. Optogenetics is considered as an advanced biological technique in neuroscience which is able to control the activity of genetically modified cells by light. Recently hDPSCs are being considered by researchers because of high potential of differentiation to neurons.
Materials and Methods: The hDPSCs were isolated by mechanical enzymatic digestion from an impacted third molar and cultured in DMEM/F12. The cells were infected with lentiviruses carrying CaMKIIa-hChR2(H134R). Opsin-expressing hDPSCs were plated at the density of 5×10^4 cells/well in 6-well plates and optical stimulation was conducted with blue light (470 nm) pulsing at 15 Hz, 90% Duty Cycle and 10 mW power for 10 s every 90 minutes for 5 days. Morphology of cells were examined daily. Two control groups including native hDPSCs and opsin-expressing hDPSCs with no optical stimulation were also included in the study. A day after last light stimulation, the viability of cells was analyzed with the MTT assay and the expression of Nestin, as a neural progenitor marker, was examined by immunocytochemistry.
Results: Human DPSCs expressed the reporter gene, mCherry, 72 hours after lentiviral infection. The result of MTT assay revealed a significant more viability in optical stimulated opsin-expressing hDPSCs as compared with two control groups. The immunocytochemistry data showed an increase in Nestin expression in optical stimulated cells compared with controls.
Conclusion: Optogenetics stimulation through depolarizing the hDPSCs can increase the cells viability and/or proliferation and also promote the differentiation toward neural progenitors.
Keywords: Optogenetics, Human Dental Pulp Stem Cells, Neurogenic Differentiation

Ps-68: Management of Full-Thickness and Non-Healing Diabetic Ulcers Using Novel Dressing based on Amniotic Membrane Extract; Preclinical and Case Study

Momeni M[1*], Azimi Alamoti M[1], Hajimiri MH[2], Jamshidi M[3], Asghari MH[4], Sodeifi N[5], Shayan Asl N[7], Dinarvand R[2, 6], Ebrahimi M[1, 7]

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

2. Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
3. Firoozgar Clinical Research Development Center (FCRDC), Iran University of Medical Sciences, Tehran, Iran
4. Animal Core Facility, Reproductive Biomedicine Research Center, Royan Institute for Animal Biotechnology, ACECR, Tehran, Iran
5. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
6. Faculty of Pharmacy, Nanotechnology Research Center, Tehran, Iran
7. Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: mebrahimi@royaninstitute.org

Background: Appropriate dressing for full-thickness and non-healing diabetic ulcers is important to shorten the wound closure time.
Materials and Methods: Because of safety and effectiveness of amniotic membrane, as well as anti-inflammatory, anti-scarring, anti-bacterial and promotion of epithelialization, we developed a wound dressing contained amniotic membrane extract loaded in PVP-chitosan gel or used as a drop.
Results: Our results indicated that AME loaded in chitosan hydrogel enhanced K1, K10, and K14 expression, increased endothelial migration and newly formed blood vessels that expressed higher CD31 gene with proper thickness of epithelial layer and organization of collagen fibers in full-thickness models in the rat as pre-clinical study phase. Application of AME as drop to treat non-healing foot ulcers was safe and efficiently accelerates wound healing in patients. However, more studies need a higher number of patients to approve the efficacy of the component.
Conclusion: In conclusion, this study introduces AME in treating chronic wound treatment. The simple production, easy to use by patients, cost-effective, room temperature storage of AME makes it a valuable biological component for marketing.
Keywords: Amniotic Membrane Extract, Re-Epithelialization, Wound Healing, Chitosan

Ps-69: Microscopic Changes of Lung Inflammation in Pre-Clinical Stem Cell Therapy

Mokhber Dezfouli MR[1, 2], Jabbari Fakhr M[2], Sadeghian Chaleshtori S[1, 2], Noori A[3]
1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2. Institute of Biomedical Research, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Email: mjabbarifakhr@yahoo.com

Background: Acute respiratory distress syndrome (ARDS) is diffusive damage to the pulmonary parenchyma. Animal models limitation of ARDS has disclosed the pathogenesis and pathophysiology of this clinical syndrome. Although progress in understanding the pathogenesis of ARDS and in intensive care medicine has been satisfactory, the treatment approach is still depending on the diagnosis of the clinical view. Applying these models have evaluated different novel treatment approaches for ARDS. There are many different reason which can initiate and induce ARDS. Animal experiments seem invaluable tools for exploring the pathogenesis and for evaluating novel treatment approaches to ARDS.
Materials and Methods: Experimental model of ARDS were induced by intratracheal delivery of LPS in 10 males white New Zealand rabbits. Then, in the treatment group BM-MSCs were isolated and cultured and 107 cells were autographed and PBS was injected in the control group intratrachealy. The rabbits were sacrificed seven days after transplantation. Then, the thoracic cavity was cut, and the lungs was ligatured, dissected and removed from the chest. Sections of the lungs were fixed and the process was routinely done.
Results: Sections of the lung shows severe histopathological patterns in the control group compared to the treatment group as hemorrhage in parenchyma and alveoli, moderate to severe vascular hyperemia and interstitial pneumonia, severe alveolar injuries and edema, neutrophilic margination in the capillary vessels, abundant presence of inflammatory cells, epithelial cells and other cell debris in interstitial spaces and alveoli and thickness of inter-alveolar septum. But in the treatment group, BM-MSCs reduced the infiltration rate of inflammatory cells in intra-alveolar, hyperemia, hemorrhage and edema, and also lungs structure were approximately normal and the thickness of the alveolar septum were slightly increased.
Conclusion: We demonstrated that MSCs could attenuate the inflammation by reducing pathological lung changes.
Keywords: Rabbit, Histopathology, MSCs, ARDS

Ps-70: The Effects of Human Menstrual-Derived Stem Cells Transplantation on Premature Ovarian Failure in Rats

Noory P

Department of Regenerative Biomedicine, Cell Science Research Center, Tehran, Iran
Email: noory.parastoo@yahoo.com

Background: Premature ovarian failure (POF) is one of the problems that young women faced during chemotherapy. POF is a common disorder caused by depletion of the ovarian reserve, which results in menopause and infertility. Among the various treatment methods, the use of mesenchymal stem cells is recognized as a suitable treatment. Mesenchymal stem cells have therapeutic potential and can be derived from several sources, including bone marrow, adipose tissue, amniotic fluid and etc. All of them show the potential for restoring ovarian function and save long-term fertility in female mice treated with chemotherapy. But the method of collecting these cells is highly invasive so it is difficult to use in the clinic. Recently, the population of highly proliferative stem cells in menstrual blood were detected with similar properties of mesenchymal stem cells and are expressed without restrictions.Various studies have been done on the therapeutic function of HuMenSCs in various diseases. This study examine the effect of menstrual blood stem cells on the improvement of function and ovarian regeneration in POF models.
Materials and Methods: Female Rats were injected intraperitoneally with 36 mg/kg busulfan. HuMenSCs were obtained, grown and analyzed for immunophenotypic features at passage three. The cells were labelled with CM-Dil and infused into the rats, 7 days after i.p. injection of busulfan for homing assay. There were four groups: normal, negative control (the rats were administered busulfan), treatment (rats were injected intravenously with 1×10^6 autologous HuMenSCs in a volume of 1mm PBS) and Sham, (rats were injected intravenously with

1mm PBS via the tail vein). One month after treatment, the ovaries were collected and weighed. Histological sections were prepared from the ovary and HuMenSCs were tracking. Then the follicles were counted and classified. Ovarian function was evaluated by monitoring ovulation. Also Histological sections were stained for TUNEL test. Subsequently, we examined the changes of expression of BAX and BCL2 apoptotic genes by Real-time PCR assay.
Results: One week after injection of busulfan, the ovaries were atrophied and evacuated from the follicle. Cultured HuMenSCs indicated a high level of expression of CD44 and CD90 but low levels of CD34 and CD45 (for all $P \leq 0.05$). One month after HuMenSCs transplantation, these cells were located in the ovarian interstitium and Granulosa cells and the number of follicles and ovary weight increased. Apoptosis was evaluated by Tunnel staining and the expression level of Bax and BCL2 genes. The number of TUNEL-positive cells significantly decreased in treatment group ($P<0.0001$). And also the expression level of Bax genes significantly decreased compared to negative and sham groups ($P<0.0001$). There was also an increase in the level of BCL2 gene expression in the treatment group.
Conclusion: HuMenSCs improve and restore ovarian function and reduce apoptosis in damaged ovarian tissue caused by busulfan toxicity. Since access to these cells is easy and non-invasive, therefore the use of these cells can be a practical and low-cost method for the treatment of POF patients.
Keywords: POF, Humenscs, Busulfan, Apoptosis, Granulosa Cells

Ps-71: Manufacturing of Allogenic GMP-Compliant Cell Bank from Human Bone Marrow-Derived Clonal Mesenchymal Stromal Cells for Clinical Applications

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

1. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: Pakzad_m@ymail.com

Background: Mesenchymal stem/stromal cells (MSCs) with two intrinsically comprehensive characteristics, low immunogenicity and smart multi behavior (pro- and anti-inflammatory) in response to signals, have been broadly manufactured and utilized in numerous clinical trials. Due to the heterogeneous nature of MSCs and upcoming senescence, allogeneic therapies have not been used as widely as autologous ones. Mainly, autologous therapy raises costs and time of remedies. Moreover, quality, scalability, and sustainability as other key elements of successful manufacturing, have been missed largely with autologous strategy.
Materials and Methods: In this study, we developed a method based on the subfractionation culturing method (SCM) to separate non-adherent mesenchymal progenitors (NAMPs) as more potent and proliferative MSCs in good manufacturing practice (GMP) environment to overcome the aforementioned hurdles. Notably, to reduce the overall costs, four banking steps were designed including seed stock, initial, master and working cell banks (ICB, MCB, and WCB). After picking up colonies in primary culture of bone marrow and subculture until passage 3, cells were frozen in 2-3 vials as seed stock. A small amount of each colony was passaged to reveal passageable ones in 11-12 more serial passages. Passageable colonies from seed stock were thawed to establish ICB, MCB, and WCB at P5 ± 1, P10 ± 1, and P15 ± 1, respectively. Each step has passed it necessitate assays, however, in MCB in addition to biological properties (morphology, immunophenotype, differentiation, senescence, growth curve and doubling time), functional potentials (cytokine secretion, immunosuppressive effect and migration assays) were also considered as determinants for screen colonies to fit specific diseases. Moreover, in WCB biosafety concerns (immunogenicity, genetic stability, and tumorigenicity) were considered as release tests to establish the end of production cell bank (EoPCB).
Results: In the end, we reached to three colonies that meet all characteristics and potentially could cure more than 5,000 patients with average cell demand of 1.5×10^8 cells.
Conclusion: Therefore, our GMP-compliant cell bank could potentially support many clinical trials with low cost, high quality and sustainable sources from a small amount of aspirated BM.
Keywords: Cell Banking, Cell Production, Good Manufacturing Practice, Mesenchymal Stem/Stromal Cells, Quality Control

Ps-72: Growth Differentiation Factor-6 (GDF-6) Induce Tenogenic Differentiation of Equine Adipose-Derived Mesenchymal Stem Cells (Eq-Ascs)

Parham A[1, 2*], Javanshir Sh[1], Naderi Meshkin H[3]

1. Department of Basic Sciences, Veterinary Faculty, Ferdowsi University of Mashhad, Mashhad, Iran
2. Stem Cell Biotechnology and Regenerative Medicine, Institue of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Stem Cell and Regenerative Medicine Research, ACECR, Mashhad, Iran
Email: parham@um.ac.ir

Background: Improvement of musculoskeletal differentiation of mesenchymal stem cells (MSCs) (both *in vitro* and in vivo) is an interesting fields in stem cell research. In order to use stem cells for treatment of orthopedic disorders especially tendon injuries, using different growth factors are recommended. The aim of this study was to evaluate differentiation of eq-ASCs in response to growth differentiation growth factor-6 (GDF-6).
Materials and Methods: Characterized and frozen eq-ASCs from 3 horses were thawed and were cultured to passage 4 (P4). Then, P4 cells were subjected to differentiation for a period of 14 days in a culture medium containing the GDF-6 factor (20 ng/ml) as treatment group. In control group, medium was the same as treatment group except inducing factors and GDF-6. After 14 days, tenogenic differentiation was evaluated by H&E and Sirius red staining for the morphological assessment and measurement of collagen production. In addition, the expression of specific genes including sclerosis (Scx), collagen type 1 (ColI α1) and Mohawk were evaluated using qPCR method.
Results: H&E staining revealed a stretching and spindle shape (tenocye-like) of cells in the treatment group. Sirius red staining data showed a significant increase in collagen production in treatment group compared with control group. Based on qPCR data, expression of Mkx, Scx and ColI was significantly increased in the treatment group compared to control group.
Conclusion: The results of this study showed that GDF-6 could induce tenogenic differentiation in eq-ASCs.
Keywords: GDF-6,Mesenchymal Stem Cells, Tenogenic Differ-

entiation, Horse

Ps-73: Donor Affect Tenogenic Differentiation of Equine Adipose Mesenchymal Stem Cells ASCs

Parham A[1, 2*], Younesi Soltani F[1], Naderi Meshkin H[3]

1. Department of Basic Sciences, Veterinary Faculty, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Stem Cell Biotechnology and Regenerative Medicine Research, Institue of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Stem Cell and Regenerative Medicine Research Center, ACECR, Mashhad, Iran
Email: parham@um.ac.ir

Background: The advent of regenerative medicine has brought us the opportunity to regenerate, modify and restore organs function. Stem cells, a key resource in regenerative medicine, can generate into one or more specialized cell types like tenocytes. Before clinical application of stem cells, their characteristics should be determined. This study designed to investigate weather donor has effect on tenogenic differentiation of equine adipose mesenchymal stem cells (ASCs) in response to platelet-derived growth-factor (PDGF).
Materials and Methods: Equine ASCs derived from three horses were used in this experiment. ASCs at passage 5 were cultured with basic culture medium containing PDGF-BB (20 ng/ml), L-Prolin and ascorbic acid (AA) at 37°C under 5% CO2 for 14 days. The cells in control group, were cultured with only basic culture medium. At the end, Sirius Red and H&E staining were used to assess collagen production, as a extracellular matrix protein for tendon, and cell morphology.
Results: ASCs in treatment groups were changed to elongated spindle shape like tenocytes. The results of Sirus Red staining revealed that the production of collagen has been elevated in treatment groups compared with control groups significantly. Statistical analysis showed that the production of collagen is different among ASCs derived from 3 horses.
Conclusion: Our data revealed that cell differentiation toward tenocytes was different for each horses. In other words, donor affect tenogenic differentiation potential of equine Ascs.
Keywords: Equine, Mesenchymal Stem Cells, Donor, Tenogenic Differentiation

Ps-74: Study of Differentially Expressed miRNA in Rhabdomyosarcoma

Radak M[*], Ghamari N, Zolfaghari AP, Fallahi H

Department of Biology, Razi Medical University, Kermanshah, Iran
Email: mehranradak@yahoo.com

Background: MicroRNAs have recently emerged as powerful post-transcriptional regulators of diverse cellular processes with especially important roles in disease and tissue remodeling, with about ~21 nucleotides in length. MiRNAs are divided into two major groups, OncomiR and Tumor Suppressor that they control many cellular processes in cancers. In addition, miRNAs have roles in extracellular transportation. This concept makes them an important potential therapeutic option. In this study, we identify the differentially expressed miRNA (DE-miRNA) in the Rhabdomyosarcoma (RMS) and create a network for them.
Materials and Methods: At first, we used GSE69524 dataset (GPL20275 platform), which information is freely available in the NCBI database. The lists were further filtered to remove the miRNAs with a P value ≤ 0.001. After that, submit miRNAs 0f Rhabdomyosarcoma in the mirawalk site and the target genes of these miRNAs were predicted. In order to identify the DE-miRNA, we must compare the predicted target genes and differentially expressed genes (DEGs) of Rhabdomyosarcoma together, to obtain the DE-miRNA.At the end, the resultant network was visualized and analyzed in Gephi v 0.9.2 software.
Results: We conclusion from obtaining information that there are 18 DE-miRNA in Rhabdomyosarcoma, which 8 of them have been upregulated and 10 of them have been downregulated.
Conclusion: Four of these DE-miRNA have not yet been investigated. This information can help us to find new therapies and drugs against Rhabdomyosarcoma cancer.
Keywords: Rhabdomyosarcoma, miRNA, Mirawalk, Therapeutic

Ps-75: Evaluation of Differentiation of Spermatogonial Stem Cell Derived Non-Obstetric Azoospermic Men in Media with Vitamin C Differentiation Factors *In Vitro*

Rafiee Z[*], Saki Gh

Department of Anatomical Sciencea, Molecular and Biological Research Center, Ahwaz, Iran
Email: ghasemsaki@yahoo.com

Background: Spermatogonial cells (sscs) are responsible for spermatogenesis throughout life in the male.because disruptions of spermatogenesis *in vitro* in order to treat infertility is essential. Thise study was aimed to investigate isolation and enrichment of adult human SSCs, compare the in-vitro effects of collagen and gelatin with growth factors on the proliferation of SSCs and then induction *in vitro* differentiation of sscs with sertoli cells coculture and hormones in presence and absence of vitamin C.effects of the culture condition on differentiation and expression of the genes involved in spermatogenesis was evaluated as well as apoptosis
Materials and Methods: TESE samples of nonobstructive azoospermic(NOA) patients, was dissociated with enzymatic digestion to obtain a cell suspention. The CD49f+ cells, as a marker to identify spermatogonial stem cells, were sorted using fluorescence-activated cell sorting (FACS). Isolated testicular cells were cultured IN dmem supplemented with GDNF, EGF and LIF in the presence and absence of collagen and gelatin coated-dish. Colony assay was performed during culture. Presence of spermatogonia in colonies was determined by an immonucytochemistry based on OCT4 and α6-integrin expression. On the other hand, sertoli cells were isolated by Lectin DSA ad culture.For *in vitro* differentiation, the cells that cultured in previous step cultivated on sertol cells in present of hormones with and without vitamin C after 4 weeks.After 2 and 4 weeks the cells ere collected in in different groups and the effects of supplementary media on the differentiation induction and expression of the meiosis and postmeiosis genes including C-KIT, SCP3, TH2b. PRM and acrosin were evaluated by RT-PCR and immunocytochemistry. Also after 2and 4 weeks apoptosis of cells was assessed by annexin V/P1 and flow cytometery
Results: SSCs enrichment was up to 75% .the findings indicated that the addition of GDNF, EGF and LIF on collagen-coated dishes significantly increased *in vitro* spermatogonial cell number and colony formation in comparsion with the other group.

The expression of spermatogonial markers include OCT4 and α6-integrin was maintained throughout the proliferation culture period.C-KIT, SCP3,TH2b,TP1,PRM and Acrosin were expressed in both with and without vitamin groups.expression of genes in vitamin group was better than without vitamin groups. Viabillity invitamin group was more than withoutvitamin group (P<0.001) and present study indicate that CD49f is a suitable marker for SSCs isolation.
Conclusion: Furthermore concluded that human SSCs obtained From NOA patients had the ability to self-renew in the culture system and collagen coated dish with growth factors can be used for the propagation of a small number of these cells from small biopsies. Coculture of SSCs Obtained from non-obstructive azoospermic (NOA) Patients with sertoli cells in presence of hormones and vitamin C play an important role in meiosis induction and maintain of SSCs. Our systems may provide new window to treatment of maile infertility.
Keywords: Spermatogonia, Azospermi, Vitamin C

Ps-76: Fabrication and Characterization of Injectable Thiolated Gelatin-Gelma Hydrogel for Internal Surgery

Rajabi Dehnavi N*, Kharaziha M, Emadi R, Soltani S

Department of Materials Engineering, Isfahan University of Technology, Isfahan, Iran
Email: rnegar29@gmail.com

Background: Recently, different kinds of adhesive surgical sealants have been used for healing or regenerating of the ruptured tissues. Between them, hydrogel-based bioadhesives have been widely applied as alternatives for sutureless wound closures. However, most of them reveal various disadvantages such as low adhesion, unsuitable mechanical strength and cytotoxicity concerns.
Materials and Methods: To overcome the challenges facing with previous sealants, a novel smart hydrogel with in-situ gelation property was prepared based on gelatin. Due to good biocompatibility, biodegradability, nonimmunogenicity and accessibility gelatin based materials have been widely used in different studies for surgical sealants. In this research, two types of modifications were applied on the structure of gelatin consisting of thiolation and methacryloyl process.
Results: Results demonstrated that compared to methacrylated gelatin (GelMA), the mechanical strength of hybrid hydrogel consisting of thiolated gelatin and GelMA was significantly increased (upon 6 times). Moreover, the hybrid hydrogel revealed less than 10% energy loss in five-cycle compression test.
Conclusion: It could be concluded that the combination of injectability, rapid mechanical recovery with at least energy loss and appropriate mechanical properties of hybrid hydrogel could make it appropriate as a sealant for internal surgeries.
Keywords: Thiolated Gelatin, Gelma, Wound Healing Application

Ps-77: Drug Repurposing: Deep Learning Approach Through Data Integration Pubmed Case Study Metformin

Rezaei Z[1*], Eslami B[2], Ebrahimpour-Komleh H[1,4], Chavoshinejad R[2], Totonchi M[3]

1. Department of Computer and Electrical Engineering, University of Kashan, Kashan, Iran
2. Department of Computer Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Mabna Veterinary Labaratory, Karaj, Alborz, Iran
Email: totonchimehdi@gmail.com

Background: Biomedical text mining has become very crucial because of increasing number of related documents. Alongside with the development in machine learning, obtaining good knowledge from literature has become popular in academia, and deep learning has enhanced the progress in efficacy of text mining models in medical sciences. However, deep learning models need a lot of training data, and application of deep learning to biomedical text mining fails frequently because of not having training data in medical sciences. Current discoveries on training contextualized language representation models on text corpora clarifies on the likelihood of benefitting from a lot of unannotated medical text corpora. Development in welfare and diet have created noticeable enhancement of life expectancy around the world. Our knowledge about scientific ground these morbidities has been quickly improved, however, successful new medication has not been created or discovered, yet. From that, alternative drug development approaches like the reposition of already known drugs for treatment of other diseases, are now being explored. This would save a lot of time and money because the pharmacokinetics, pharmacodynamics and safety profiles of these drugs have been already characterized, In fact drug reprofiling effectively would get around preclinical studies, required for newly invented medication. Metformin is one of medication on which there has been ongoing research on its new application Previous clinical evidence show that metformin has been normally prescribed for diabetes treatment. This study aims at investigation into the possible effects of this medication on various diseases, published in PubMed. In other words, we studied the reported results, available in medical literatures for potential of metformin to prevent or treat different kinds of disorders.
Materials and Methods: We look for relevant publication in Pub Med through using metformin as key word. This search cover studies have been done between 1994 a d 2019, which was written in English language. In this direction, we applied NER BioBERT method. Bidirectional Encoder Representations is transformation of Text Mining and specific area of language representation model, useful in wide range of texts. According to this structure, BioBERT actively relocate the information from a lot of biomedical texts to biomedical text mining models by a few modifications in task specific structure. While BERT illustrate excellent function with former models, BioBERT noticeably overtake them on entity recognition and clustering with regard to metformin impact on individuals' health. Different disease and the trend of metformin impact is in publication during several years, based on drug effect on various disease
Results: There has been some reported investigation into the association between metformin and the results of remedy in various diseases. In addition to these preclinical report, reliable biological pathways have been known which explain the molecular mechanism of metformin and addressed in our study. However, the important answer to this question that the level of metformin efficacy against non-diabetic disorders. Up until there is not clear answer for that in clinical trial, the role of metformin on treatment or prevention of disease remains hypothetical.
Conclusion: Up until there is not clear answer for that in clini-

cal trial, the role of metformin on treatment or prevention of disease remains hypothetical that in this paper has been investigated all of their dimensions through clustering and NER in PubMed.

Keywords: Drug Repurposing, Deep Learning, Metformin, Pubmed, Biobert

Ps-78: Human Pluripotent Stem Cell-Derived Erythrocyte Production Using Small Molecule Chir99021

Sabahi Moosavi N[1*], Barati M[1, 2], Janzamin E[2], Baharvand H[2], Hassani SN[2]

1. Department of Cell and Developmental, 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
Email: nargess.sabahi@yahoo.com

Background: Human pluripotent stem cells (hPSCs) including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have made it possible to produce manipulated hematopoietic stem cells (HSCs), erythrocytes, and lymphocytes. Specially, iPSCs has the potential to provide the personalized blood cells like universal erythrocytes which can be used to study hematologic disorders and new treatments. Several hPSC-derived erythrocyte production protocols have been introduced based on using feeder (from human or mice) cells and human plasma. Here we defined a feeder-free hematopoietic differentiation system by using small molecule CHIR99021, a GSK3β inhibitor and cytokines replicating hematopoiesis during development.
Materials and Methods: In this study hematopoietic differentiation in hPSCs was inducted by CHIR99021 in hypoxic condition. Hematopoietic stem/progenitor cells (HSPCs) released from hemogenic sacs after further induction by VEGF, IL-6, IL-3, bFGF, erythropoietin (EPO) and SCF, were then cultured in MethocultH4434 to measure colony forming capacity and the erythroid colonies were then counted. Cells were also characterized by RT-PCR, Wright-Giemsa staining and immunofluorescence and flow-cytometry.
Results: Induction of definitive hematopoiesis pathway in hPSCs and hypoxia result in hemogenic sac-like structures. These hemogenic sacs release CD43+ CD45+ hematopoietic progenitor cells capable of producing erythroid colonies after being cultured in semi-solid methyl cellulose based media. Our data showed that hESCs produced more erythroid colonies than iPSCs (22.5% over 9.5% of total colonies). After being in cytokine enriched condition for two weeks, these colonies indicated CD235a+ erythroblasts which were nucleated and showed different stages of maturation by Wright-Giemsa staining.
Conclusion: Our findings indicate that erythrocytes can be produced from HSPCs produced in our defined protocol. While having fetal characteristics, showing different cell shapes and low yield that can pose as an obstacle in clinical approaches, these hPSCs-derived erythrocytes can still be used as a source to study and improve our knowledge of erythrocyte development and the *in vitro* drug delivery systems as they are not affected by cell number and their lower affinity toward oxygen.

Keywords: Erythrocytes, Human Pluripotent Stem Cells (hPSCs), Hematopoietic Stem Cells (HSCs), Hematopoietic Stem/Progenitor Cells (HSPCs)

Ps-79: Wnt/β-catenin Signaling Pathway Enhances Early Dopaminergic Differentiation of Trabecular Meshwork-Derived Mesenchymal Stem Cells.

Sahebdel F[1*], Zeynali B[1], Parvaneh Tafreshi A[2], Arefian E[3]

1. Department of Cell and Developmental Biology, University of Tehran, Tehran, Iran
2. Department of Basic Sciences in Biotechnology, National Research Institute of Genetic Engineering and Biotechnology, Tehran, Iran
3. Department of Microbiology, University of Tehran, Tehran, Iran
Email: zeynalib@ut.ac.ir

Background: Parkinson's disease is the second most common neurodegenerative disorder. Degeneration of dopaminergic neurons in the Substantia nigra causes the loss of dopamine related functions. So far Wnt/β-catenin signaling pathway is shown to be involved in differentiation of dopaminergic neurons. Our preliminary results in the present study showed that the neural crest-derived trabecular meshwork MSC (TM-MSC) could be an appropriate source to differentiate into neurons. Therefore, we sought to examine neural differentiation potential of these cells compared to the two other sources of MSCs including adipose-derived (ADMSCs) and bone marrow (BMSCs) mesenchymal stem cells and to determine the role of Wnt/β-catenin signaling pathway in early dopaminergic differentiation.
Materials and Methods: To address these questions, the cells were cultured in the presence and absence of neural induction medium for six days and analyzed by real time PCR and immunofluorescence staining. To examine the effect of Wnt/β-catenin signaling on dopaminergic differentiation, CHIR (Wnt agonist; 3 μM) and IWP-2 (Wnt antagonist, 3 μM) were used.
Results: Our morphological analysis showed that TM-MSCs exhibited a better neural characteristic than ADMSCs and BMSCs. Also, more enhanced expression of Nurr-1, the early dopaminergic marker, was present in TM-MSCs compared to ADMSCs and BMSCs. In the neurally induced TM-MSCs, there were significant increased expression of Nurr-1 and MAP2 compared to those in the non-induced control cells. A combined treatment with CHIR and the neural induction medium, further enhanced the expression of Nurr-1 and MAP2 as well as those of the Wnt/β-catenin signaling target genes, c-Myc and Cyclin D1. Conversely, a combined treatment with IWP2 and the neural induction medium significantly decreased the expression of Wnt/β-catenin target genes and the neural markers.
Conclusion: Altogether, we suggest that TM-MSCs have a higher neural potency than other commonly used MSCs and can potentially be induced towards the dopaminergic differentiation via the activation of Wnt/β-catenin pathway.

Keywords: Wnt/β-catenin, Dopaminergic,Trabecular Meshwork

Ps-80: Self-Organization of Liver Organoids in 3D Liver Extracellular Matrix-Derived Hydrogele

Saheli M[1, 2, 3*], Sepantafar MM[4, 5], Pournasr B[5], Farzaneh Z[5], Vosough M[5], Piryaei A[2], Baharvand H[3, 5]

1. Department of Anatomical Sciences, School of Medicine, Afzalipour University of Medical Sciences,Kerman, Iran
2. Department of Cell Engineering, Cell Science Research Center, 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. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technology in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
6. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: baharvand@royaninstitute.org

Background: An important advantage of employing extracellular matrix (ECM)-derived biomaterials in tissue engineering is the ability to tailor the biochemical and physical microenvironment of the cells. This study aims to assess whether three-dimensional (3D) liver ECM-derived hydrogel (LEMgel) promotes physiological functions of self-organized liver organoids generated by human hepatocarcinoma cells together with human mesenchymal and endothelial cells.
Materials and Methods: We have fabricated LEMgel with the greatest content of glycosaminoglycans, collagen, laminin and fibronectin. During gelation, complex viscoelasticity modulus of the LEMgel (3 mg/ml) increased from 186.7 to 1570.5 Pa and Tan Delta decreased from 0.27 to 0.18. Scanning electron microscopy (SEM) investigation determined that the LEMgel had a pore size of 382 ± 71 µm.
Results: The self-organized organoids in 3D LEMgel (LEMgel-organoid) expressed ALB, CYP3A4, E-cadherin, and ASGPR. The LEMgel-organoid had significant upregulation of transcripts of ALB, CYP3A4, CYP3A7, and TAT as well as downregulation of AFP compared to collagen type I- and scaffold-free-organoids or organoids in the LEM emulsion and 2D culture of hepatocarcinoma cells. Generated 3D LEMgel-organoids had significantly greater ALB and AAT secretion, urea production, CYP3A4 enzyme activity, and inducibility.
Conclusion: In conclusion, liver organoid self-organization within the 3D LEMgel promoted hepatocyte function compared to traditional 2D, 3D, and collagen gel cultures. Our novel 3D LEMgel-organoid could potentially be used in liver tissue engineering, drug discovery, or toxicology studies.
Keywords: ECM-Derived Hydrogel, Human Hepatocarcinoma Cells, Liver Organoid, Tissue Engineering

Ps-81: Ccar1 as A Partner of B-Catenin Correlates with Ground-State Pluripotency in Mescs

Samadian A*, Taleahmad S, Molamohamadi S, Baharvand H

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

Background: Dual inhibition of extracellular signal-regulated kinase (MEK) and transforming growth factor beta (TGFβ), known as R2i, helps to maintain the mouse embryonic stem cells (mESC) under-ground state condition. To understand the molecular mechanisms of R2i, in our previous study we investigated the proteome profile of mES cells that was cultured under R2i condition. According to the proteomics data, the protein expression level of the cell cycle and apoptosis regulator 1 (Ccar1) as a partner of β-catenin, was significantly upregulated in R2i culture.
Materials and Methods: In this experimental study, we used siRNA against Ccar1 and then we analyzed β-catenin localization and its target genes expression by immunofluorescence assay and qRT-PCR, respectively.
Results: The Immunofluorescence data showed that the reducing Ccar1 level by siRNA influenced the β-catenin translocation. The qRT-PCR analysis revealed significant downregulation of the pluripotency genes as well as some cell cycle markers such as Ccar1, c-myc and Tbx3 in the siRNA treated cells. In addition, the expression level of the Wnt target genes such as Cdx1, Wnt3a, Tbx1, Fgf4, Apc and Cdh1 was significantly upregulated by the knockdown of Ccar1.
Conclusion: In conclusion, upregulating of Ccar1 under R2i culture condition, can prevent β-catenin nuclear translocation and maintains the pluripotency and self-renewal of mESCs.
Keywords: MESCs, Pluripotency, β-catenin, Ccar1, Wnt Signaling

Ps-82: Upregulated of Hsa-Mir-625-5p Inhibited Invasion of Cancer Cell AML through ILK/AKT Pathway

Samieyan Dehkordi S[1*], Mousavi SH[1], Ebrahimi M[2, 3], Alizadeh Sh[1], Hedayati Asl AA[2, 3], Mohammad M[3], Aliabedi B[1]

1. Department of Hematology, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran
2. Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Emails: hmousavi@tums.ac.ir, mebrahimi@royaninstitute.org

Background: Acute myeloid leukemia is a malignant disorder characterized by abnormal growth and differentiation of primary hematopoietic stem cell and leads to accumulation of immature myeloid precursors in the blood and bone marrow. The spread of immature myeloid cells is accompanied by the normal production of other differentiated cells, including red blood cells and platelets. In many studies, miR-625 has been shown to inhibit the downstream pathways involved in the invasion and metastasis of the integrin –linked kinase(ILK) signaling pathway, and also it has been proved that expression of miR-625 has decreased in acute myeloid leukemia cell lines. Therefore, the increase expression of miR-625 may be caused by induction of apoptosis, reduction of metastasis and invasion in cancer cells of acute myeloid leukemia by reducing the associated oncogenes. The aim of this study was to investigate the effect of miR-625 on invasion via the ILK pathway in KG-1 cell line. In order to investigate the mechanism of miR-625 effect on KG1 cell invasion, the important oncogenes of the ILK pathway were investigated.
Materials and Methods: The KG1 cell line was transfected with a vector containing pLenti-miR-625-5p-GFP and pLenti-Backbone-GFP by viral method. Then, confirming the increase of miR-625 expression by q-RT-PCR, the amount of cell invasion in the laboratory environment was assessed by invasion assay. Finally, in order to investigate the mechanism of miR-625 effect, the levels of COX2, NF-ÁB, GSK3, MMP9, AP1, AKT, ILK at the gen level by q-RT-PCR and on the protein level were evaluated proteins NF -κB and MMP-9 were investigated by western blotting. Also, CXCR4 levels were assessed by flow cytometric as invasive and homing cell markers.
Results: Investigation of invasive KG1 cells transfected by miR-625-5p structure showed a significant decrease in the

invasion. ILK gene expression in these cells significantly decrease compared to the control group (Backbone). The expression of NF-κB, COX2 showed a significant decrease compared to the control group, and expression of MMP9, AP1 and AKT increased significantly, whereas GSK3β did not change significant. Changes in the expression of MMP9 and NF-κB protein were observed so that level protein of NF-κB is decreased and MMP9 don't have significant change. To evaluate the expression of CXCR-4 by flow cytometry, the expression of CXCR4 in the miR-625 group was significantly lower than that of the control group.
Conclusion: Increasing the expression of miR-625-5p leads to decrease cellular invasion in AML cells and can be considered as a new strategy to help treat AML patients in the future. However, further studies are needed to achieve this goal.
Keywords: Invasion, miR-625, Acute Myeloid Leukemia, ILK, MMP9 and COX2

Ps-83: Effect of Human Umbilical Cord Perivascular Cells on Improving Functional Recovery Following Cerebral Ischemia/Reperfusion Injury

Seifali E[1*], Mahdavipour M[1], Hassanzadeh Gh[1], Moini A[2], Akbari M[1]

1. Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Gynecology and Obstetrics, Roointan Arash women's Health Research and Education Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: akbarimo@tums.ac.ir

Background: Ischemic stroke occurs due to obstruction of one of the main arteries of the brain. This situation can lead to neuronal death. Tissue plasminogen activator (TPA) is only accepted treatment for acute ischemic stroke. Mesenchymal stromal cells (MSCs) display therapeutic successfulness. The human umbilical cord perivascular cells (HUCPVCs) have the potential for brain regeneration and protection after injury. The purpose of this study was to investigate the effect of HUCPVCs on functional recovery and neuroprotection.
Materials and Methods: Ischemia was induced by middle cerebral artery occlusion (MCAO) of male wistar rats. Animals classified to Sham, MCAO, MCAO+HUCPVCs groups. Treatment carried out 2 hours after ischemia. Adhesive removal test carried out for evaluation of functional recovery. The mRNA and protein expression of MAP2 were assessed in the cerebral cortex. Nissl staining performed on histological sections to evaluating the number of dead neurons in the ischemic boundary zone (IBZ).
Results: Our results demonstrate that HUCPVCs administration noticeably improved functional recovery following MCAO. The number of dark neurons decreased significantly in HUCPVCs therapies group compared to the MCAO group 7th day post-ischemia ($p < 0.05$). HUCPVCs enhanced the neuronal density of MAP2 in the IBZ.
Conclusion: In conclusion, our study revealed that the HUCPVCs improved sensorimotor function possibly through reduction of dead neurons and increasing density of mature neurons after cerebral ischemia.
Keywords: Ischemia, HUCPVCs, MCAO, MAP2, Dark Neurons

Ps-84: Investigate the Link Between Abdominal Obesity and Lung Cancer: Based on Transcriptomic Data and Genome-Scale Metabolic Model

Sepahi MM[*]

Departments of Embryology and Stem Cells, Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: m.m.sepahi@ut.ac.ir

Background: Obesity is one of the critical health issues worldwide, and the evidence base for a link between obesity and cancer is growing. There is emerging evidence that general obesity and/or abdominal obesity is associated with increased risk of certain types of cancers, including breast cancer, colorectal, endometrial, esophagus, kidney, pancreatic, thyroid, gallbladder and lung cancer. Several meta-analyses of observational studies have been performed to examine the association between abdominal obesity and lung cancer. Systems biology approaches, based on the integration of various data types leading to computational and mathematical modeling, are particularly suitable for investigating the underlying molecular mechanisms of complex diseases.
Materials and Methods: The Genome-scale metabolic model of the human adipocyte, "iAdipocytes1809", was downloaded from the Human Metabolic Atlas database in the compressed Systems Biology Markup Language (SBML) format. Gene expression data were extracted from the dataset GSE78958 deposited at the Gene Expression Omnibus portal. Raw data, in CEL format, were imported, corrected, transformed and normalized through "GEOquery" and "Affy" R packages.
Results: In this work, we exploited gene expression data of lung cancer obese and lean patients to simulate a curated genome-scale metabolic model of the human adipocyte, available in the Human Metabolic Atlas database. We performed a network topology differential analysis to search for pattern differences and identify the principal reactions associated with significant changes across the two conditions under study. In particular, we show that the qualitative and quantitative assessment of metabolic fluxes modulated by gene expression data provides a valuable method for investigating the mechanisms associated with the phenotype under study, and can foster our interpretation of biological phenomena.
Conclusion: As a case study, we investigated the relationship between abdominal obesity and lung cancer. In summary, abdominal obesity may play an important role in the development of lung cancer.
Keywords: Abdominal Obesity, Lung Cancer, Genome-Scale Metabolic Model

Ps-85: Quality System and Regulatory Requirements for Stem Cell Banking: A Case Study for Mesenchymal Stem Cells

Sepahi MM[*], Kazemnejad S

Departments of Embryology and Stem Cells, Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: m.m.sepahi@ut.ac.ir

Background: Stem cells are highly promising resources for application in advanced therapy medicinal products (ATMPs) and drug discovery. That's why stem cell banks have been increasingly established all over the world in order to use in basic and translational research, as well as current and future clinical ap-

plication. Research to ensure the quality, safety and efficacy of the final cell preparations intended for transplantation, national and international guidelines and regulations have been issued. Herein the implementation of a quality assurance system including the principles of Good Manufacturing Practice (GMP), Quality Risk Management (QRM) and a Quality Control (QC) system is one major concern. Quality assurance system regulations apply to all phases of cell collection, processing and storage as well as premises and equipment, documentation, personnel training, quality control and the compliant handling. QC measures have to be taken to ensure that products have consistent potency, purity, safety and quality.
Materials and Methods: A risk based approach and quality by design paradigm was used to define the Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) in mesenchymal stem cells proliferation and banking. Risk Analysis and Mitigation Matrix (RAMM) was created and used to provide a parametric compromise where other risk tools.
Results: The RAMM tool was applied to an example mesenchymal stem cells banking process. The tool was applied in six stages as part of a risk management system. The first step was selecting the right team to be involved in the process development, product development, analytical method development, manufacturing and facilitator. Then CQAs was defined as the following: viral contamination, identity, viability, cellular count, sterility, mycoplasma, endotoxin, morphology and karyotype. Process steps and material was defined in next steps. The RAMM was created and used a matrix of process input factors and quality attributes to assess risk and impact. The final step was to assist in defining which action would help mitigate risk for these process parameters.
Conclusion: As a case study, we investigated the risk based mesenchymal stem cells banking process. However, strict regulations during banking processing have to be followed in order to guarantee the highest quality and safety for the patients.
Keywords: Mesenchymal Stem Cells, Risk Analysis, Good Manufacturing Practice

Ps-86: Testing Probiotics for Their Ability to Promote Pancreatic Beta Cell Regeneration Using A Microscale In Vivo Model of Diabetes Based on Transgenic Zebrafish

Shahbazi N[1, 2, 3*], Crawford A[3, 4], Lotherington L[3], Sørum H[3], Tahamtani Y[2], Hosseini F[1], Ayyari M[5]

1. Department of Biological Sciences, Alzahra University of Tehran, Tehran, Iran
2. Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway
4. Faculty of Agriculture and Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Tehran, Noor, Iran
5. Department of Horticultural Science, Tarbiat Modares University (TMU), Tehran, Iran
Email: yasertahamtani@royaninstitute.org

Background: Diabetes – both type 1 and advanced type 2 – is a disease characterized by an absolute or relative deficiency of pancreatic beta cells. Therefore, replenishing the lost beta cell function or absolute beta cell mass is a strategy to alleviate some of the burdens of the disease. One of the possible approaches to replenish beta cells is the induction of endogenous regeneration. It has been observed that probiotics modulate the microbial composition of the gut and can also modify host nutrient metabolism and energy homeostasis. The microbiome is known to influence the progression of T1DM, and altering the microbiome may therefore be a strategy to delay onset or manage T1DM pathogenesis. Three main targets have emerged for probiotic therapy in T1DM: reduction or redirection of autoimmunity, increased β-cell proliferation, and decreased β-cell apoptosis.
Materials and Methods: The zebrafish pancreas shares a basic structure and cellular makeup with the mammalian pancreas, implying that the developmental lessons we learn in zebrafish will be broadly applicable. The ability to manipulate resident microbes in the larval zebrafish, combined with the optical transparency and sophisticated genetic tools of the zebrafish model, make it a powerful platform to investigate this question. In our ongoing biodiscovery program to identify bacterial secondary metabolites with pancreatic beta cell regeneration-promoting activity, we are using a transgenic zebrafish model of T1DM. This model is based on Tg(ins:cfp-nfsb) larvae with pharmacologically inducible cell-specific ablation of pancreatic beta cells by the prodrug nifiprinol, which is metabolized by bacterial nitroreductase to cause apoptosis in pancreatic beta cells.
Results: After incubating larvae with each probiotic strain for 96 hours, fluorescence and confocal microscopy verified the regeneration of beta cells in the larvae of each experimental group and in comparison with positive and negative controls. Using this bioassay, we have to date identified at least one of our probiotic strains as having ability to increase beta cell mass. Active strains and their selected primary metabolites, e.g. short-chain fatty acids (SCFAs) will next be screened and analyzed using qRT-PCR of beta cell markers, immunohistochemistry analysis and a free glucose assay in order to verify both the function and the identity of regenerated beta cells.
Conclusion: Additional tests in higher animal models will help determine which of these probiotics and their metabolites have therapeutic potential for T1DM. Research in animal models and humans has provided evidence suggesting possible signaling pathways that can be influenced by probiotics and their metabolites, triggering proliferation and function of pancreatic beta cells directly and indirectly by production/fermentation of short chain fatty acids (SCFAs) and gamma amino butyric acid (GABA), and by stimulating the secretion of incretin hormones such as GLP-1 from entero-endocrine L-cells. Towards this end, further research will focus on the characterization of the signalling pathways involved in the regeneration of beta cells stimulated by our active probiotic strains.
Keywords: Type 1 Diabetes, Probiotics, Zebrafish, Glucagon-Like Peptide-1 (GLP-1), Short Chain Fatty Acids (SCFAs)

Ps-87: Cis pT231-tau Mediates Neurodegeneration upon Pollution

Shahpasand Sh[1*], Karima S[4, 2], Ghobeh M[1], Jahanbazi Jahan-Abad A[4, 2], Salari AA[3], Salmani F[4, 2], Shahpasand K[3]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Clinical Biochemistry, Faculty of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran
3. Department of Brain and Cognitive Sciences, Cell Science Research Center, Royan Institute
for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: karima@sbmu.ac.ir

Background: Alzheimer disease is a neurodegenerative disorder and sixth leading cause of death globally. AD is a multifactorial disease in which genetical background as well as environmental factor play role in it. Among environmental factors, pollution is of crucial importance considering modern life style. Despite extensive considerations, it remains uncertain how pollution mediates neurodegeneration and AD. There are two major pathological hallmarks playing part in AD, including miss-processed amyloid precursor protein and hyperphosphorylated tau. Tau is a microtubule associated protein the functions of which are thought to be controlled by phosphorylation. Anyhow, tau hyperphosphorylation or some post phosphorylation modifications reflects tau pathogenicity. Recent studies have demonstrated that phosphorylated tau at Thr231 exists in two distinct cis and trans conformation in which cis pT231-tau is highly neurotoxic and acts as an early driver of tauopathy in several neurodegenerative diseases. We herein studied if Cis pT231-tau has a contribution in neurodegeneration caused by pollution.
Materials and Methods: We employed mice model to examine the effect of pollution on the CNS. We studied the mouse brains using immunoflourescent staining and western blotting with conformation specific antibody against cis pT231-tau
Results: Fluorescent imaging and western blotting analysis of mouse brains demonstrated a prominent cis p-tau accumulation in pollution treated mouse models compare to control healthy ones.
Conclusion: It has been clearly demonstrated that pollution would result in tauopathy but the actual molecular mechanism has remained uncertain thus far. We have demonstrated that pollution would cause cis pT231-tau accumulation in neurons, resulting in neurodegneration. Our findings unravel tauopathy mysteries upon pollution and would help us find a right therapeutic target to fight the devastating disorder caused by modern life.
Keywords: Cis pT231-Tau, Neurodegeneration, Pollution

Ps-88: Human Embryonic Stem Cells Derived Mesenchymal Stem Cells Conditioned Medium for Chondogenic Induction of Human Embryonic Stem Cells

Shahriary Sh[1, 2*], Farashahi Yazd E[1, 3], Hajizadeh F[1], Golzadeh J[1], Tahajjodi SS[1, 3], Akyash F[1, 3], Aflatoonian B[1, 2, 3]

1. Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Advanced Medical Sciences and Technologies, School of Paramedicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Reproductive Biology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: b.aflatoonian@ssu.ac.ir

Background: Osteoarthritis (OA) is one of the most common joint diseases in the world. Cell therapy considered as a novel approach for OA treatment. Human embryonic stem cells (hESCs) because of their unlimited self-renewal and innate pluripotency, can be useful source for human articular cartilage regeneration and future OA cell-based therapies. Recently, it was shown that conditioned medium (CM) from meneschymal stem/stromal cells (MSCs) can induce chondrogenic differentiation of hESCs. Here, the chondcogenic induction of hESCs-derived mesenchymal stem/stromal cells CM (hESCs-MSCs CM) was investigated in a 3D culture for 4 days.
Materials and Methods: CM from Yazd2 (hESCs; 46,XY) derived MSCs was collected and stored at -20ºC. Embryoid body (EB) formation from Yazd4 (hESC line; 46,XX) was done as 3D culture in spontaneously differentiation (SD) and conditioned medium (CM) groups for 4 days. Subsequently, EBs from both groups were collected and RNA extraction, C-DNA synthesis and RT-PCR was performed for the β-ACTIN, MEOX1, COL2A1, RUNX2 genes.
Results: Our data indicated that after 4 days, the EBs in CM group expressed all the genes which were studied for chondrogenesis of hESCs, although, the EBs in SD group expressed none of them.
Conclusion: Our data shows that hESC-MSC CM induces chondrogenic differentiation to hESCs as shown by the gene expression data using RT-PCR. This finding opens new insights for better understanding of cellular, molecular, developmental and environmental factors in human chondrogenesis for further studies in developmental biology, toxicology and drug discovery to cure OA.
Keywords: Chondrogenesis, Conditioned Medium, Embryoid Body, Human Embryonic Stem Cells, Mesenchymal Stem/Stromal Cells

Ps-89: Three-dimensional Printing of Composite Scaffolds for Bone Tissue Engineering

Shams A[1, 2*], Masaeli E[1]

1. Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Cell Science Research, Isfahan, Iran
2. Department of Tissue Engineering, Islamic Azad University, Advanced Materials Research Center, Isfahan, Iran
Email: elahe.masaeli@royaninstitute.org

Background: Today, the need for the bone substitutes transplantation in patients how suffer from critical-size bone defect is a significant concern. Tissue engineering is a complex multidisciplinary strategy which offers a promising approach for fabrication of tissue and organ replacements. Among various techniques for fabrication of tissue-engineered scaffolds, three-dimensional (3D) printing is well suited to produce tissue-like constructs especially for bone regenerative therapies. As a case study, we designed and fabricated a composite bone scaffold by a fused deposition modeling (FDM) printer and subsequently investigated physico-chemical properties of 3D printed constructs for the purpose of using as bone replacement.
Materials and Methods: 3D poly-lactic acid (PLA) scaffolds were made by a FDM printer in a resolution of 200µm and then coated with biphasic calcium phosphate (BCP) nano-powder and collagen protein. Physico-chemical characterizations consisting scanning electron microscopy (SEM) imaging, mechanical compression test, X-ray diffraction (XRD) analysis and attenuated total reflection (ATR) spectroscopy were performed to investigate the potential use of designed scaffolds for bone tissue engineering.
Results: SEM images showed printed scaffolds possess a porous structure with suitable inter-connectivity for bone regenerative therapies. Young's modulus of scaffolds was around 97 MPa, respectively. ATR and XRD maps also represented presence of BCP and collagen on the surface of PLA network, confirming successful dip coating process after printing.
Conclusion: FDM 3D printers offers a promising approach for

fabrication of tissue-like scaffolds especially for bone regenerative therapies. In the present study, combination of a polymeric scaffold (i.e. PLA) with a bio-ceramic (i.e. BCP) and an extracellular matrix protein (i.e. collagen) successfully performed and led to the formation of a scaffold with appropriate physicochemical and mechanical properties for bone tissue engineering. Further investigations are recommended for biological assessment of 3D composite scaffolds, *in vitro* and in vivo.

Keywords: Tissue Engineering, Bone, Scaffolds, 3D printing, FDM

Ps-90: *In Vitro* Simulation of Native Biological Condition of Cardiomyocytes

Shams Z[1*], Rajabi S[2], Akbari B[1], Aghdami N[3]

1. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
2. Department of Cell Engineering, Cell Science Research Center, 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
Email: nasser.aghdami@royaninstitute.org

Background: Cardiac tissue engineering is one of the most promising procedures for heart regeneration and its main goal is to mimic the body's natural tissue with the significant role of scaffolds and mechanical simulation. Using an eslectrospun aligned nanofiber scaffold similar to the cardiac Extracellular Matrix (ECM) and applying a mechanical loading in the direction of aligned nanofibers, the expression of contraction-related genes in Cardiac Progenitor Cells (CPCs) which have been seeded on the scaffold, would be increased.
Materials and Methods: In this study, an aligned Polycaprolactone (PCL) / Gelatin nanofiber scaffold was produced with a ratio of 70 to 30 respectively. The hydrophilicity and strength of the scaffold were examined with the contact angle test and mechanical strength analysis. The homogenization of the nanofibers and cell viability also have been analyzed with scanning electron microscopy (SEM) images and MTS assay. Then to specify the effect of contraction simulation, a developed Mechanical Loading Device (MLD) applied mechanical stress with the frequency of 1Hz and 10% strain rate in direction of the aligned nanofibers in order to provide a dynamic platform for seeded CPCs. After 5 days, the quantitative reverse transcriptase PCR (qRT-PCR) analysis evaluated the expression of the genes responsible for synchronizing beating cells (MYH-6, TTN, and CX-43) in the dynamic scaffold compared to the control sample with static culture system.
Results: Our results from the chemical and physical characteristics of the scaffold, showed us that the scaffold with the contact angle of 46.96 and ultimate tensile strength at 17% tension could be undergone the mechanical loading. The qRT-PCR analysis demonstrated that contraction related genes had been expressed higher in dynamic sample indicating an appropriate transfer of tension force through CPCs.
Conclusion: Based on this study, by simulating the native cardiomyocytes condition in-vitro, the genes that are responsible for synchronized cardiac contraction have been expressed in the higher level in the analyzed CPCs. So, these cells would be a suitable candidate for transplantation to the damaged heart tissue without the possibility of developing the arrhythmia.

Keywords: Cardiac Tissue Engineeering, Aligned Electrospun Scaffold, Mechanical Simulation, Polycaptolactone-Gelatin Composite Polymer, Cardiac Genes

Ps-91: Microfluidic Approaches for Separation of Mouse Tumoral Cells from Neonate Spermatogonial Cells Suspensions

Shams A*, Ashtari Kh, Esmaeilzadeh N, Tanbakoei S, Koruji M, Mohajer Ansari J, Ebrahimi Warkiani M, Shabani R

Department of Anatomical Sciences School of Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: shabani.r@iums.ac.ir

Background: Some male survivors of childhood cancer are azoospermic. spermatogonial stem cells(SSC) isolation and purification are really important.The aim of this study was separating the mouse malignant cell line (EL-4) and SSC *in vitro*.
Materials and Methods: SSC were isolated 60 neonatal mice were used and co-cultured with EL-4 and divided into 2 groups: 1) Control (co-culture in culture medium), 2(Co-cultured cells were separated by microfluidic device. The percent of cells were assessed by flow cytometry and immunocytochemistry and confirmed by RT-PCR.
Results: The percentage of EL-4 and SSC after isolation was collected at two outlets, the output for smaller outlet was for SSC (0.12%) and EL-4 (42.14 %) While in larger outlet was SSC (80.38 %), EL-4 (0.32%) in control group, the percentage of cells were SSC (21.44%), EL-4 (23.28 %).
Conclusion: The present study demonstrates the use of electrophoretic Microfluidic device is effective in separating cancer cells from spermatogonial stem cells.

Keywords: Microfluidic Device, Spermatogonial Stem Cell, Mouse Cancerous Cell, Cell Sorting

Ps-92: Utilizing miRNA-143 and miRNA -206 Capsulated in PLGA on Induction of Apoptosis in EL4 Cancerous Cells

Shams A*, Koruji M, Shabani R

Department of Anatomical Sciences School of Medicine, Iran University of Medical Sciences,Tehran, Iran
Email: a.midwifery@gmail.com

Background: One in 250 cases of childhood cancer reaches adulthood, Increasing the likelihood of survival until adolescence creates the need for reproduction. According to statistics, more than 20-30% of these children have oligospermia in puberty. More than two-thirds of the parents of cancerous children want to freeze the testicular biopsy in the hope of a child's future fertility. This method has so far been tested in animals and has had successful results. But the major concern in this way is the possibility of biopsy tissue contamination with non-tumor cells, Today, many micro RNAs are used to induce apoptosis in cancer cells. In this study, the effect of transfection of micro RNAs 143 and 206 on apoptosis induction in cancer cells was investigated.
Materials and Methods: EL4 Cancer cells were purchased from the Pasteur Institute, in order to encapsulate micro-RNA in the PLGA polymer structure, emulsion-solvent penetration was used. Surface functionalization of micro RNAs -loaded PLGA NPs is done with folate to better targeting. In order to

obtain optimal dose from each micro, several doses based on previous studies were evaluated in the dosimetry stage. So that cells {EL4} were treated separately at 3 doses {for micro RNA 143 doses of 25,50 and 75 ng and for micro RNA 206 doses of 50,100 and 150 ng}.
Results: The experiments were performed three times. Based on MTT the best dose of mir 143 was 75 ng EL4 viability (45%), and for mir 206 was 150 ng EL4 viability (28%), and when both of them was used EL4 viability was 37%.
Conclusion: PLGA-Capsulated microRNA is a new method for apoptotic induction in cancer cell to achieve the pure cell after biopsy and healthy transplantation.
Keywords: Nanoparticle of PLGA, Micro RNA, Apoptosis

Ps-93: Effect of human Adipose Stem Cell Derived Exosomes on Proliferation and Neural Differentiation of PC12 cells

Shariati Najafabadi S*, Amini Sh, Salehi H, Amirpour N

Department of Anatomy, Isfahan University of Medical Sciences, Isfahan, Iran
Email: samira.sharyaati@gmail.com

Background: The nervous system has limited regeneration capacity. So researchers are always looking for an appropriate treatment for neurodegenerative diseases. Exosome treatment improves the therapeutic outcomes and prevents the concerns of cellular and surgical therapies that are due to the capability of exosomes to start the internal repairing processes of damaged tissues and modulate the immune responses. The aim of this study was to investigate the effect of human Adipose Stem Cell derived exosomes on proliferation and neural differentiation of PC12 cells.
Materials and Methods: Following the isolation of hASCs exosomes, TEM, DLS, Bradford and Flow cytometry techniques were used to confirm successful isolation. These exosomes were used for proliferation and differentiation of PC12 cells. Entrance and effect of exosomes assessed by Acridine orange staining, MTT assay and ICC technique
Results: This study present that Adipose Stem Cell derived exosomes can enhance cell viability of PC12 cells and promote neural differentiation and expression of mature neural markers in these cells
Conclusion: Our results propose that hASCs-exosomes may facilitate the way for the non-cellular treatment of neurodegenerative diseases.
Keywords: Exosome, Adipose Stem Cell, Proliferation, Differentiation

Ps-94: Differentiation Induction and Proliferation Inhibition by A Cell-Free Approach for Delivery of Exogenous miRNAs to Neuroblastoma Cells by Mesenchymal Stem Cells

Sharif Mashadi S[1*], Ghahremani MH[2], Soleimani M[3]

1. Department of Molecular Medicine, Tehran University of Medical Science, Tehran, Iran
2. Toxicology and Pharmacology, Tehran University of Medical Science, Tehran, Iran
3. Department of Heamatology, Tarbiat Modares University, Tehran, Iran
Email: sharif_s@razi.tums.ac.ir

Background: Neuroblastoma (NB) is one of the most frequent malignant solid tumors in infancy and childhood, accounting for 15% of pediatric cancer deaths. Defective differentiation in neural crest cell precursors of sympathetic nervous system causes NB. Recently, the approach of differentiation therapy has shown considerable promise ineffective treatment of NB patients. MiR-124 is a kind of nervous system-specific miRNA that increases during neuronal differentiation and may be one of the potential therapeutic targets in the treatment of NB. However, despite its well-established therapeutic potential, its efficient delivery to the targeted tumor cells is a challenging task. Mesenchymal stem cells (MSCs) are multipotent adult progenitor cells that are also reported to have antitumor effects and the capability of migrating toward cancer cells and tumors
Materials and Methods: In this study co cultured hAD MSCs and M17 NB Cells, then after 72 hours, the M17 NB cells were collected and flow cytometric analysis was used to detect the delivery of miR-124-Cy3 to M17 cells. Apoptosis induction in the treated M17 cells with transfer miR-124 and control miR was measured using the TUNEL kits.
Results: In this study, it is shown that human adipocyte-MSCs (hAD-MSCs) have the ability to deliver exogenous miRNAs to NB cells. A specific kind of miRNA, i.e. miR-124, is successfully delivered with hAD-MSCs to M17 NB cells via direct or indirect (exosome-based) contacts. It is demonstrated that the indirectly delivered miR-124 significantly decreases the proliferation of NB cells and induces their differentiation.
Conclusion: These results offer the opportunity to use the delivered exogenous miRNAs by the derived exosomes from hAD-MSCs as a novel cell-free stem cell-based therapy for NB cancer.
Keywords: Neuroblastoma, MiR-124, Exosome, Mesenchymal Stem Cells, Differentiation

Ps-95: An Efficient Method for Expression and Purification of Active Human IGF-1 in Escherichia Coli

Siavashi S[1, 2*], Aboutalebi F[1], Nasr Esfahani MH[1], Khayam Abed R[2, 3], Dormiani K[1]

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

Background: The generation of neurons in the adult mammalian brain requires the activation of quiescent neural stem cells (NSCs). This activation and the sequential steps of neuron formation from NSCs are regulated by a number of stimuli such as growth factors. Insulin-like growth factor-1 (IGF-1) is one of these factors that exerts pleiotropic effects and regulates multiple cellular processes based on its concentration, cell type and the developmental stage of target cells. This factor shows trophic effects on neuronal regeneration in the central and peripheral nervous systems. It stimulates protein synthesis in neurons, glia, oligodendrocytes and Schwann cells that result in inhibition of cell apoptosis. Human IGF-1 is a non-glycosylated polypeptide hormone containing 70 amino acids in a single chain with three intramolecular disulfide bonds. Recently, several variants of engineered human IGF-1 have been produced in order to improve the protein stability, activity and tighter binding

to IGF-1 receptor. However, the major challenges in production of recombinant human IGF-1 are the yield, purification and the activity of produced peptide as well as the cost of production. In this study, an efficient method was employed to produce active human IGF-1, which was purified without any solubilizing tag.
Materials and Methods: The gene of tobacco etch virus (TEV) protease and coding sequence of hIGF-1 with a His-tag, which was fused to TEVsite and Trx (Thioredoxin) gene were cloned into two cloning sites of pET-Duet vector under control of tac and T7 promoters respectively. The vector was transformed into SHuffle T7 competent E.¬coli and expression condition was optimized for expressing active TEV and Trx/TEVsite/His-tag/hIGF-1 fused protein simultaneously. Afterwards, one-step purification procedure was employed to purify His-tag/hIGF-1 using Ni-NTA resin. Western blot was used to confirm the accuracy of the purified protein and its functionality was examined on NIH3T3 cells.
Results: Our results demonstrated that TEV protease digested its specific site between Trx and hIGF-1 in bacterial cells and the soluble His-tag/hIGF-1 was extracted without Trx-tag. Followed by, the effect of purified protein on NIH3T3 cells proliferation showed that the recombinant protein was functional and folded correctly.
Conclusion: This research developed an efficient method to produce soluble and active recombinant hIGF-1 in E.¬coli, which was purified without any solubilizing tag using a simple, feasible procedure.
Keywords: Neural Stem Cells (Nscs), Insulin-Like Growth Factor-1 (IGF-1), Tobacco Etch Virus (TEV) Protease, Purification, Recombinant

Ps-96: Design and Fabrication of Injectable Alginate Hydrogels by Guest-Host Assembling

Soltani S[1*], Emadi RA[1], Haghjoo Javanmard Sh[2], Kharaziha M[1]

1. Department of Materials Engineering, Isfahan University of Technology, Isfahan, Iran
2. Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Email: samaneh.soltani34@yahoo.com

Background: Injectable hydrogels afford direct injection or catheter delivery to tissues. However, many injectable hydrogels require long reassembly times or the use of triggers such as chemical initiators or heat.
Materials and Methods: We developed an injectable alginate (Alg) hydrogel based on the guest-host interactions of adamantine modified Alg (guest macromer, Ad-Alg) and β-cyclodextrin modified Alg (host macromer, CD-Alg).
Results: The ability of conjugation to Alg was confirmed by 1H NMR spectroscopy, Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffraction (XRD). Mixing of Ad-Alg and CD-Alg resulted in rapid formation of a hydrogel composed of guest–host bonds.
Conclusion: The guest–host assembly mechanism allowed both shear-thinning behavior for ease of injection and near instantaneous reassembly for material retention at the target sight. These hydrogels show potential as a minimally invasive injectable hydrogel for biomedical applications and tissue engineering.
Keywords: Injectable Hydrogel, Guest-Host Interactions, Alginate, β-Cyclodextrin, Tissue Engineering

Ps-97: Critical Functions of MicroRNAs during Embryonic Stem Cell Derivation

Soori S[1*], Khoddami V[2], Braun Th[3], Baharvand H[2], Moradi Sh[2]

1. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center of Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Cardiac Development and Remodelling, Max-Planck Institute for Heart and Lung Research, Germany

Email: sharif.moradi@gmail.com

Background: Embryonic stem cells (ESCs), derived from inner cell mass (ICM) of pre-implantation embryos, exhibit unlimited self-renewal and multi-lineage differentiation potential, which is due to activity of a variety of biomolecules, including microRNAs. Here, for the first time, we functionally analyzed specific candidate microRNAs during ESC derivation under R2i condition (dual inhibition of Fgf/Erk and TGF-β pathways).
Materials and Methods: Immunosurgery was performed to isolate ICMs from blastocysts and culture them under R2i condition to generate ESC lines. MicroRNA profiles were obtained using small-RNA sequencing during ICM-ESC transition (Moradi et al, unpublished data). MicroRNA mimics were transfected into ICM to explore their effects on ESC derivation, or into ESCs to examine if they influence ESC self-renewal. Candidate upregulated microRNAs were analyzed in the presence of Fgf/Erk inhibitor (PD032, with a low ESC derivation efficiency) to assess if they promote ESC generation, while downregulated candidates were studied in R2i culture (with ~100% derivation efficiency) to examine if they impair ESC derivation. Alkaline phosphatase (AP) staining was performed to analyze ESC self-renewal.
Results: Small-RNA sequencing revealed that microRNAs exhibited dynamic expression patterns. We considered certain criteria e.g. dynamic and abundant expression, conservation, and previously documented functions to shortlist the microRNAs down to 25 candidates, mostly reported to be involved in regulating pluripotency. Among these, miR-212-5p and let-7d-3p (with downregulated patterns) and miR-183-5p and miR-363-3p (with upregulated patterns) appeared to be the best candidates. Then, we examined the impact of these microRNAs on the efficiency of ESC derivation, and found interestingly that miR-212-5p and let-7d-3p significantly decreased the number of ESC lines derived in R2i culture compared to the scrambled control. Notably, the majority of ESC colonies in miR-212-5p and let-7d-3p-treated groups were partially differentiated. On the other hand, miR-183-5p and miR-363-3p considerably enhanced the efficiency of ESC derivation in the presence of PD032, compared to the scrambled oligo.
Conclusion: MicroRNAs downregulated during ICM-ESC transition impair ESC derivation and those upregulated during this process improve the efficiency of ESC formation.
Keywords: ESC, Inner Cell Mass, Embryonic Stem Cell, Microrna, Ground State Pluripotency

Ps-98: Evaluation of The Dip-Coating of Ibuprofen on Poly(Lactic-Co-Glycolic Acid) Microneedles

Tabatabaei SA[1*], Nejabat GR[1], Shokrolahi F[2], Khademi F[3]

1. Department of Polymer Engineering, Shiraz Branch, Islamic Azad University, Shiraz, Iran
2. Biomaterials, Iran Polymer and Petrochemical Institute (IPPI), Tehran, Iran
3. Department of Tissue Engineering and Cell Therapy, Shiraz University of Medical Sciences, Shiraz, Iran
Email: ghnejabat@yahoo.com

Background: Dip-coating method is one of the popular methods for loading drugs on microneedles. A rapid drug delivery can be achieved by drug coated microneedles through formation of pores in the stratum corneum of the skin. Since pain relief drugs usually need to be absorbed quickly, microneedles coated with these types of drugs seems to be an attractive system for delivery of them to the body. The purpose of this study is to evaluate the loading of ibuprofen (as a model of analgesic drugs) on a poly (lactic-co-glycolic acid) (PLGA) microneedle patch through dip-coating technique.
Materials and Methods: PLGA powder (molecular ratio of D, L-Lactide/glycolide 50:50, Mw 20000 Da) was provided by IPPI; ibuprofen was donated by Aryapharm Company (Tehran, Iran) and methanol was bought from Merck (Germany). Specific amounts of ibuprofen were dissolved in methanol to give a clear solution. Then the PLGA microneedle that was prepared through a melt-molding technique was dipped into the solution such that the needles were in direct contact with the solution. FTIR and DSC were used to confirm the loading of the drug on microneedles. Finally SEM was used to monitor the morphology of the needles before and after the drug loading process.
Results: FTIR analysis confirmed the loading of ibuprofen on PLGA microneedles and the results showed that the drug was loaded mainly through physical absorption. The results were also confirmed by Differential scanning calorimetry (DSC) through showing characteristic transitions of PLGA and the drug. Morphology investigations through SEM before and after coating process as well as penetration of microneedles into para-film (as a skin model) showed that the shape and morphology of the microneedles after the drug coating is preserved while after penetrating into the film the sharpness of a few number of microneedles were lost.
Conclusion: The results showed that the process of ibuprofen loading on PLGA microneedles was successfully performed through dip-coating without any sign of deformation in the morphology of needles. Some minor changes in the tip of the needles were observed after penetrating into para-film.
Keywords: Microneedle Patch, PLGA,Dip-Coatong, Ibuprofen, Loading

P-99: Long Term p38 MAPK Inhibition Endows Endoderm Propensity to Human Embryonic Stem Cells

Taei A[1, 2], Samadian A[2] , Mola Mohammadi S[2] , Ghezel Ayagh Z[1, 2], Kiani T[2] , Moradi Sh[2], Hassani SN[2], Baharvand H[1, 2*]

1. Department of Developmental 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
Email: adeleh.taei@gmail.com

Background: Inherent biological properties of human embryonic stem cells (hESCs) including differentiation into any somatic cell type and self-renewing have opened up numerous opportunities in biomedical research and cell replacement therapies. Under suitable culture conditions, hESCs can be stabilized. However, these cells have the ability to sense and response to various environmental signals. Extrinsic fluctuation in their environment could direct cell death or differentiation in hESCs. P38 mitogen activated protein kinases (p38-MAPK) is a signaling pathway that is activated with a variety of environmental stress and plays a role in cell differentiation, apoptosis, and autophagy. Nevertheless, P38-MAPK function on hESCs is remained unclear.
Materials and Methods: Here, we suppressed p38-MAPK in different hESC lines (Royan H5 and Royan H6) by using a small molecule inhibitory, SB203580 (10 µm), in continuous passages. After a few passages a series of stemness features were evaluated. The results were compared with non-treated hESCs as a control group.
Results: We showed that p38-MAPK inhibition resulted in changing morphology of hESCs from flat form to small and dome-shaped. These cells displayed a high growth rate, increased clonogenicity, and were resistance to single cell dissociation by trypsin relative to non-tread hESC. Furthermore, we found that some naïve specific genes were upregulated in treated-hESC with p38-MAPK inhibitor such as; NANOG, STELLA, KLF2, KLF5, TFCP2L1, and DNMT3L. These cells simultaneously expressed pluripotency and lineage specific markers. Spontaneous differentiation showed that P38i-treated cells efficiently expressed AFP and ALB genes which are representative for hepatocyte differentiation. In addition, we detected higher percentage of endodermal like cells in teratoma formed by p38i-treated hESCs. We also found more than two fold increase in SOX17 expression at protein level when p38i-treated cells were directly differentiated into endoderm in comparison with non-tread hESCs.
Conclusion: Taken together, our results suggest that suppression of p38-MAPK in hESCs could provide a cell resource for robust endoderm cells in order to facilitate in mature and reliable hepatocyte and pancreases cell differentiation.
Keywords: Human Embryonic Stem Cells, p38 MAPK Pathway, Endoderm Differentiation

Ps-100: Decellularized Osteochondral Sheets as a Bioscaffold Support Mesenchymal Stem Cell Proliferation and Differentiation in A Rabbit Model

Taghiyar L*, Asadi H, Bagaban Eslaminejad MR

Department of Stem Cells and Developmental Biology, Cell Science Research Center, oyan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: leilataghiyar@royaninstitute.org

Background: Decellularized osteochondral tissue is believed to be the best natural extra cellular matrix (ECM) for restoration of the osteoarthritis defects. Biomechanical feature, presence of differentiation-stimulating signals, preserved connection of bone-to-cartilage border, and existence of a tissue-specific structure are the most valuable innate properties of osteochondral tissue to be used as a bioscaffold as compared to artificial ones. Despite the advantages, osteochondral is a compact skeletal tissue with low porosity particularly in the cartilaginous segment which causes whole-organ decellularization, cell-seeding and subsequent cell-penetration challenges coming forward. This study aimed to develop a new construct of decellularized osteochondral tissue incorporated by bone marrow derived-mesenchymal stem cells (MSCs), as an osteochondral allograft,

for preserving the cartilage–subchondral bone interface integration.

Materials and Methods: For this purpose, whole osteochondral tissues were collected from rabbit knee joints; then, cartilaginous parts were sheeted in 200- 250 µm sections while connected to subchondral bone and were fully decellularized. The MSCs were derived from rabbit bone marrow, cultured and expanded by successive subcultures and characterized. Passage-3 cells were then seeded into the bioscaffold and incubated for 3 weeks; Moreover, some cell-derived-ECM were subcutaneously implanted into the back of the rabbit and maintained for 4 weeks.

Results: We found that constructed sheets were completely decellularized. *In vitro* and *in vivo* histological and scanning electron microscopy (SEM) assessments indicated that the cells could successfully penetrate into the bone and cartilage lacunas in implanted grafts. Importantly, gene expression analysis results indicated that seeded cells differentiated into osteoblasts and chondroblasts in both bone and cartilage segments following exposure to native ECM elements and growth factors. Further, cartilage-to-bone border integrity was largely preserved.

Conclusion: These results indicate that ECM-sheeted decellularized osteochondral constructs could be employed as a useful scaffold for promoting regeneration of osteochondral defects.

Keywords: Decellularized Osteochondral Tissue, Allograft, Mesenchymal Stem Cells, Subcutaneous Implantation, Recellularization

Ps-101: Peripheral Nerve Contribute to Repair And Regeneration of Injured Mammalian Tissue

Tahouri M

School of Biology, College of Science, University of Tehran, Tehran, Iran

Email: Mahsa.tahouri1992@gmail.com

Background: Mammals have lost multi-tissue regenerative capacity in comparison with amphibians, but they have this ability for the distal digit amputation. During regeneration of the mouse digit tip, Schwann Cell Precursors (SCPs) that exist in peripheral nerve, dedifferentiate and move into the injured tissue. Schwann cell precursors are derived from neural crest, researches indicate that neural crest-derived cells appear to respond to injury and stress by acquisition of a new cell fate in a process that likely involves dedifferentiation and in vivo reprogramming. Recent researches also showed mesenchymal cells that reside within nerves play a crucial role in mammalian tissue repair and regeneration. They identified a specific population of mesenchymal cells that represent a reservoir of precursors that expand and differentiate into bone and dermis during tissue regeneration and repair.

Materials and Methods: Immunostaining used for visualizing to report expressing PDGFRα gene. For neural crest-derived, using a mouse carrying a Wnt1-Cre transgene that is expressed in embryonic neural crest precursors and a TdTomato reporter gene with an upstream floxed stop cassette in the Rosa26 locus.

Results: It has been shown, Following digit tip removal, these dedifferentiated SCPs localize to injured tissue, where they secret oncostatin M (OSM) and platelet-derived growth factor AA(PDGF-AA), to promote self-renewal of mesenchymal cell, expansion of the blastema, and regeneration. Previous research showed that OSM also can promote osteogenic differentiation. The authors discovered that kinds of epineurial and perineurial fibroblasts exist regardless of whether the nerve is damaged or not. However, endoneurial fibroblasts change and downregulate some connective-tissue-associated genes while upregulating other genes, it highlights the hypothesis in which regenerative and repair cells are derived from endoneurium. endoneurial fibroblasts arise from neuroectoderm neural crest cells instead of the lateral plate mesoderm.

Conclusion: Finally, proposed that nerve damage causes dedifferentiation of Schwann cells and expansion of endoneurial precursor-like mesenchymal cells which then migrate into injured tissues, where they promote repair by two complementary mechanisms; the dedifferentiated Schwann cells secrete growth factors like PDGF-AA and oncostatin M and nerve-derived mesenchymal cells differentiate into tissues like the dermis and bone in response to local cues.

Keywords: Mammalian Tissue Repair,Mesenchymal Cell, Peripheral Nerve, Schwann Cell Precursors

Ps-102: Human SRY May Play A Negative Role in Conversion of Primed to Naïve Human Pluripotent Cells

Taleahmad S[1], Alikhani M[1], Taleahmad F[2], Molamohammadi S[1], Baharvand H[1, 3*], Hosseini Salekdeh Gh[4, 5]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
4. Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Department of Molecular Sciences, Macquarie University, Sydney, NSW Australia

Email: Baharvand@RoyanInstitute.org

Background: Both of primed and naïve state are pluripotent stem cells but exhibit slightly different properties. The identification of signaling pathways which differences between these two cells can be useful to the conversion of primed to naïve state.

Materials and Methods: In this study, we analyzed the expression data of the UGent11.7 cell line (XY cell line) which previously reported. By the loss of function approach, we investigated the role of Y chromosome genes under the primed state using specific siRNA treatment. The findings were validated by gene and protein expression by (qRT-PCR) and western blot respectively.

Results: We found that most of the Y chromosome genes like SRY showed high expression level in primed versus naïve hESCs. Therefore, we hypothesize that SRY prevents WNT/β-catenin signaling by interaction and inhibition of β-catenin translocation. By the loss of function approach through depletion of SRY on the primed cells, we observed that the expression of pluripotency markers and WNT signaling target genes such as Brachuary, GCBM, TBX2, and TBX3 increased in si-SRY treated cells.

Conclusion: In conclusion, we revealed that inhibition of SRY results in nucleus translocation of β-catenin and up-regulation of WNT signaling pathway which important to naïve conversion.

Keywords: Human Embryonic Stem Cell, Naïve, Primed, SRY, Wnt Signaling Pathway

Ps-103: Fabrication of a Polysaccharides Base Porous Substrate in Myocardial Tissue Engineering

Tamimi M*, Rajabi S

Soft Tissue Engineering Research Center, Tissue Engineering and Regenerative Medicine Institute, Central Tehran Branch, Islamic Azad University, Tehran, Iran

Email: sarahrajabi83@gmail.com

Background: Application of principles and approaches of engineering and life sciences to perceive fundamental and mutual structure–function relationships in native and pathological heart tissues resulting from cardiovascular diseases such as myocardial infarction and its subsequent side effect including heart failure,and to develop biological substitutes to restore, maintain, or improve tissue function were all emergence objectivity of the term "myocardial tissue engineering .From all approaches have been applied in this field so far, deriving of extracellular matrix (ECM) from different native tissue was a novel approach.
Materials and Methods: By consideration of this trend, we acellularized an animal myocardial tissue by sodium dodecyl sulphate (SDS, 1%). Its resultant fine pulverized extracellular matrix (ECM) was digested and blended with two polysaccharide-based polymers, freeze dried, and fabricated a ternate substrate for cell seeding
Results: Microscopically images of histological Characterization of extracellular matrix by Hematoxylin and Eosin, Masson's Trichome, and Alcian blue staining validated the removal of cellular content and preservation of collagen – the most ample component present in the ECM – and glycosaminoglycans (GAGS). Evaluation tests including physical, chemical and mechanical characterization of this substrate were performed at *in vitro* condition. Physical characterization such as morphology seen by scanning electron microscopy (SEM) and porosity showed porous substrate with interconnected pores and porosity more than 90%. Fourier Transform Infrared (FTIR) spectrum revealed interaction of the present functional groups of applied polysaccharide-based polymers together with digested extracellular matrix . Mechanical properties showed that with addition of polysaccharide-based polymers to the composition,unilateral ultimate tensile strength of substrates in comparison with the substrate free from polymers has significantly increased .Seeded human pelvic mesenchymal stem cells on the substrates had attachment and proliferation at defined time 1, 3 and 7 days
Conclusion: Generally,validated substrate requirements which are including to be porous structure with interconnected pores to facilitate inward and outward transportations of materials, to be biocompatible, and to have suitable mechanical property, made this substrate as a suitable matrix for subsequent hopeful application in myocardial tissue engineering.
Keywords: ECM, Substrate, Polysaccharides, Myocardial Tissue Engineering

Ps-104: Sprayable Visible-Light Kappa-Carrageenan Hydrogel for Wound Healing

Tavakoli Sh*, Mokhtari H, Kharaziha M, Kermanpour A

Department of Material Enginnering, Isfahan University of Technology, Isfahan, Iran

Email: sh.tavakoli73@yahoo.com

Background: Recently, soft tissue engineering emerged extensively all over the world due to men's need to organ transplantation and the lack of enough immune sources. Sprayable hydrogels are distinctive material for in-situ soft tissue regeneration and skin engineering.
Materials and Methods: The aim of this study was to develop sprayable visible-light crosslinked Kappa-carrageenan (κCA) hydrogel for healing wounds and investigate the role of polymer concentration (2, 4 and 6 wt.%) and degree of methacrylation (6 and 12 %) on its physical and mechanical properties. first, κCA was methacrylated by different degree and was treated using Eosin Y and triethanolamine (TEA) as the photoinitiator and co-initiator, respectively. then the samples were exposed under visible light to crosslink.
Results: It was found that, the water content (91.05-95.06%), swelling ratios (11.12-20.66%) and average pore sizes (26.28-79.13 μm) were revealed to be tunable by changing the methacrylate κCA (KaMA) concentrations as well as methacrylation degree. Furthermore, the mechanical characteristics of KaMA hydrogels could be noticeably modulated, depending on the formulation of KaMA hydrogel. For instance, the tensile and comprehensive modules were enhanced from 68.25 to 357.73 kPa and from 213.16 to 357.73 kPa respectively, by increasing KaMA concentration from 2 to 6 wt.% and methacrylation degree from 6 to 12 %. Increasing the polymer concentration and degree of methacrylation reduced the gelation time to 90 s. *in vitro* studies revealed that visible-light crosslinked KaMA hydrogels not only was biocompatible, but also could promote the attachment and proliferation of HaLa cells, depending on the KaMA concentration and methacrylation degree. Moreover, degree of methacrylation and polymer concentration affect primary hydrogel solution viscosity. Our results showed higher methacrylation degree and lower KaMA concentration decrease hydrogel solution viscosity which is more appropriate for spraying.
Conclusion: Based on these result the hydrogel containing 4% wt KaMA with high methacrylation could be applicable biomaterial for spraying with proper mechanical, physical and biological property. It could be concluded that, visible-light crosslinked KaMA is an exclusive biomaterial which can engineer as a sprayable hydrogel to cover skin injuries rapidly in-situ, by varying methacrylation degree and KaMA concentration.
Keywords: Kappa-Carrageenan, Sprayable Hydrogel, Visible-Light Cross-Linking

Ps-105: Effect of Different Concentrations of Iron Oxide Nanoparticles on Expression of p53 Gen in Human Derived Amniotic Membrane Mesenchymal Stem Cell

Tekiyeh Maroof N[1*], NaserOleslami M[1], Aboutaleb N[2]

1. Department of Cellular and Molecular Biology, Faculty of Advanced Science and Technology, Tehran Medical Sciences,Islamic Azad University, Tehran, Iran
2. Department of Physiology Department, and Physiology Research Center, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Email: naseroleslami@gmail.com

Background: Paramagnetic nanoparticles (SPIONs) have made extensive advances in nanotechnology. The unique properties of these particles have expanded their application in various fields, including medical fields. One of these uses is non-invasive analysis for cell tracking. However, the possibility of toxicity in cells is reported by these nanoparticles. Due to the

fact that cellular damage caused by iron oxide nanoparticles is concentration-dependent. So finding the appropriate concentration of iron oxide nanoparticles is very important to prevent cell damage or cell death caused by apoptosis. The aim of this study was to find a concentration of SPIONs that does not result in apoptosis in cells. Therefore, this study aimed to investigate the effects of different concentrations of iron oxide nanoparticles on cell survival, the effect on increased gene expression and proteins involved in apoptosis (P53) In human amniotic membrane derived mesenchymal stem cells (hAMSCs) were evaluated.

Materials and Methods: First, stem cells were extracted from human amniotic membrane tissue and cultured. Then, the viability of the cells treated with different concentrations of iron oxide nanoparticles (200, 150, 100, 50, 0 µg / ml) over a period of 24 hours was evaluated by MTT method. The effect of concentrations of 0, 100,150 and 200 µg / ml of nanoparticles after 24 hours in hAMSCs was investigated for expression of P53 gene by Real-Time PCR. Further, the expression of P53 protein expression in cells treated with nanoparticles Iron oxide was measured using western blot method.

Results: Iron oxide nanoparticles had no significant effect on cell survival at concentrations of 50 and 100 µg / ml in 24 hours, while cell survival was significantly reduced at concentrations of 150 and 200 µg / ml. The results of qRT-PCR analysis showed that the expression of P53 gene increased at a concentration of 150 µg / ml and above, significantly. In addition, Western Blot results showed that the expression of P53 protein also increased in cells treated with 150 and 200 µg / ml concentrations of nanoparticles.

Conclusion: According to the results, the nanoparticles used in this study are appropriate at concentrations ≤ 100 µg / ml for cell tracking.

Keywords: Amniotic Membrane Stem Cells, Iron Oxide Nanoparticles, Apoptosis, P53 Gene, Spions

Ps-106: Peripheral Blood Stem Cell Mobilization Can Be Affected by SNS Neurotransmitters

Vahdani N[1*], Tavakoli F[2], Kuhestani B[2], Sankanian Gh[2], Roshandel E[2]

1. Department of Biology, Faculty of Science, University of Tehran, Tehran, Iran
2. Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: elham.roshandel@gmail.com

Background: Hematopoietic stem cell transplantation (HSCT) has provided considerable therapeutic success for hematologic and non-hematologic diseases. Improving the efficacy of stem cell mobilization is an essential step in the prosperity of the transplantation process. Granulocyte colony-stimulating factor (G-CSF) is the most common mobilizer agent. However, the significant poor mobilize population is a convincing reason to conduct investigations into another mechanism to enhance mobilization yields. Recently, accumulating evidence has demonstrated the involvement of the nervous system in HSC mobilization. Therefore, the goal of this study is figuring out the role of donor pre-apheresis epinephrine (EPI) and norepinephrine (NE) levels in mobilization.

Materials and Methods: 20 healthy, patient-related donors were included in this prospective study. Donors treated with subcutaneous G-CSF for 4 to 6 days. Blood was collected before G-CSF treatment and before apheresis. ELISA was performed for detection of EPI and NE levels in plasma. CD34+ and CD3+ cells were counted by flow cytometry (Attune NxT, Country) with PE-conjugated human anti-CD34 (EXBIO, Czech Republic) and FITC-conjugated human-CD3 (Beckman Coulter). Statistical analyses were performed using SPSS for Windows (Version 19) (SPSS Inc., Chicago, IL, USA) to evaluate a correlation between neurotransmitter levels and CD34+ cell count in PB.

Results: Measurement of plasma levels of NE and EPI before the start of G-CSF administration was within the normal limits. The levels of CD34+ cells in PB before apheresis and apheresis product were significantly correlated with plasma levels of catecholamines. Data showed healthy donors with a higher concentration of NE and EPI have more CD34+ cells in peripheral blood. Thus, the yield of apheresis is better (P-value: < 0.05). However, the number of CD3+ cells in the apheresis product was not statistically significant (P value: 0.35).

Conclusion: The regulatory effects of the nervous system on HSC mobilization is well-defined and has been entered in the clinical setting. Moreover, given that the number of CD3+ cells was not affected by plasma levels of catecholamines, these neurotransmitters can be considered as a good HSC mobilizer in the setting of allogeneic HSCT to maximize the usefulness and limit any possible complications of mobilization.

Keywords: Epinephrine, Norepinephrine, Mobilization, G-CSF

Ps-107: Genetic and Epigenetic Indices of Spermatogonial Stem Cells *In Vitro* and *In Vivo* Groups

Zahiri M[1*], Movahedin M[2], Mowla SJ[3], Noruzinia M[4], Koruji M[5, 6], Nowroozi MR[4, 6], Asgari F[4]

1. Department of Anatomical Sciences, School of Medical Sciences, Bushehr University of Medical Sciences, Bushehr, Iran
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Medical Genetics, School of Medicine, Tarbiat Modares University, Tehran, Iran
4. Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
5. Department of Urology, Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran
6. Department of Urology, Uro-Oncology Research Center, Tehran University of Medical Sciences, Tehran, Iran
Email: movahed.m@modares.ac.ir

Background: Epigenetic changes, especially at the level of DNA methylation, play a role in the behavior of stem cells. It due to their ability to change, compared with genetic changes, is more suitable targets for new treatments. Although various protocols have been developed to human spermatogenesis; an optimal protocol is still needed to satisfy scientists. In this study, we examined epigenetic and genetic changes of various cultural systems according to their efficiency in proliferation and purification of human spermatogonial stem cells.

Materials and Methods: Obstructive azoospermia patients-derived spermatogonial cells were cultured in five different groups under special conditions for 2 weeks, and then the cells were divided into two groups. Equal number of cell population in each group was enrichened by MACS for GFR- α1 antibody and the other part was not enrichened. Gene specific methylation and quantitative genes expression of pluripotency (Nanog, C-Myc, and Oct-4) and specific germ cell (Integrin α6, Integ-

rin β1, PLZF) genes in each stage were evaluated by MSP and quantitative PCR. To reveal functionality, spermatogonial cells from the selected group were transplanted to recipient azoospermia mouse model. The immunohistochemical, morphological and genetic were evaluated.
Results: Expression of germ specific genes in testicular cell suspension and after purification were significantly increased ($P \leq 0.05$). Nanog and C-Myc expression level were significantly decreased in this group ($P \leq 0.05$). The best culture system was the culture of the testicular suspension. There was no significant difference in the expression of Oct-4 among testicular cell suspension and other groups ($P > 0.05$). Gene specific methylation pattern of examined genes did not show any changes during culture period. The use of MACs for purification of human stem cells was also effective at about 69%.
Conclusion: An examination of the epigenetic pattern showed that the specific methylation of the evaluated genes at this stage remained constant throughout the entire culture system over time and the culture conditions did not change the methylation pattern. Our results confirmed that culture of testicular cell suspension and selection of spermatogonial cells could be effective ways for purification and enrichment of the functional human spermatogonial cells and studied culture systems improved spermatogenesis *in vitro*.
Keywords: Azoospermia, Genetic and Epigenetic, Human Spermatogonial Cells

Ps-108: Effect of Leydig and Sertoli Cells Respective Culture on Expressions of Self-Renewal Biomarkers in Spermatogonial Stem Cells of Mice

Zaker FS[1*], Razi M[1], Mahmoudian AR[2], Soltanalinejad F[1]

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
2. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: Fahimeh_zaker@yahoo.com

Background: The Sertoli cells-produced Glial cell-derived neurotrophic factor (GDNF) has been known to directly induce the spermatogonial stem cells (SSCs) self-renewal by interacting with its special receptors Gfrα1 and C-Ret. Thus, the current study was designed to investigate whether testosterone, as known co-factor in maintaining and progressing spermatogenesis, plays a role in stimulating the Sertoli cells to produce GDNF or not?
Materials and Methods: Therefore, the TM4 cells (line Sertoli cells) were co-cultured with different concentrations (0.1 ng/ml, 0.2 ng/ml, and 0.4 ng/ml) of exogenous (commercial pure testosterone) and endogenous TM-3 (line Leydig cells)-produced testosterone, and consequently the TM4-produced GDNF concentration was evaluated by ELISA, immunocytochemistry (ICC) and qRT-PCR analyses. In the second step, the activity of produced GDNF was estimated by co-culturing the SSCs with the TM-4 derived media (containing endogenous GDNF). For this purpose, the SSCs were dissected (from 5-6 days old male neonates), purified, approved (using qRT-PCR of SOX-2, THY1, and NANOG), and finally cultured with TM-4-derived media (containing 0.1 ng/ml, 0.2 ng/ml, and 0.4 ng/ml of GDNF). Next, the expressions of Gfrα1 and C-Ret were evaluated by ICC and qRT-PCR. The cell viability ratios were evaluated in each step of study using the MTT test.
Results: Observations showed that endogenous testosterone, albeit in 0.1 ng/ml and 0.2 ng/ml, as well as exogenous testosterone (at 3 dose levels) could fairly up-regulate the GDNF expression versus non-treated Sertoli cells. The 0.4 ng/ml endogenous testosterone-treated Sertoli cells represented diminished expression of GDNF versus other treated cells in the same category. In continue, Observations revealed that TM-4-produced GDNF, albeit at 3 dose levels, could up-regulate the Gfrα1 and C-Ret expression. Meanwhile, this situation was more notable in 0.1 and 0.2 ng/ml GDNF-treated SSCs.
Conclusion: In conclusion, our findings showed that testosterone plays a role in stimulating the Sertoli cells to synthetizing of GDNF. Moreover, the testosterone-induced GDNF is able to amplify its receptor's expression both at mRNA and protein levels. Moreover, we found that GDNF solely is able to amplify its receptor expression may as a stimulator factor.
Keywords: Testosterone, GDNF, Gfrα1, C-Ret, SSCs

Ps-109: Morphine Treated Mesenchymal Stem Cells Conditioned Medium Enriches Cancer Stem Cell Populations of Murine Mammary Tumor 4T1 Cell Line

Zand S[*], Delirezh N, Abtahi Froshani M

Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
Email: n.delirezh@urmia.ac.ir

Background: Mesenchymal stem cells (MSCs) are a group of non-hematopoietic adult stem cells with capacity for self-renewal and differentiation. They are an important component of the tumor microenvironment and recruited by cancer cells to similarly promote tumor growth and progression. Morphine, an opiate-based agent is widely used for management of severe pain associated with cancer metastasis. Many tumors, including breast cancer, consists of undifferentiated slow-cycling cells with self-renewing capacity which maintain tumor growth called cancer stem cells (CSCs). These cells are identified with CD44+ /CD24- cell surface markers. An increasing amount of evidences indicate a possibility that morphine causes immunosuppression on the hosts. This survey was designed to determine the effect of morphine on the interaction of MSCs and 4T1 cells that may promotes CSCs populations.
Materials and Methods: MSC was isolated by flashing the Tibia and femur bones of mice. After 14 days, MSCs were incubated for 24 h with 0 and 10 µM of Morphine. Then cells were cultured without serum for 24h and the conditioned medium (CM) was isolated. 4T1 cells were incubated for 2 weeks in a medium with isolated CM (50%) and FBS. The medium was changed every 2 or 3 days. Cells were then harvested and incubated with antibodies against CD44 and CD24 for detection of CSCs.
Results: Flow cytometry analysis indicated that the CM of MSCs treated with 10 µM of morphine could significantly increases the number of CSCs within murine mammary tumor 4T1 cell line. Percentage of 10 µM is almost two times higher than percentage of control group.
Conclusion: This data clearly showed that treatment with CM of morphine treated MSCs results in enrichment of cancer stem cell populations within 4T1 cells.
Keywords: Mesenchymal Stem Cells, Conditioned Medium, Morphine, 4T1 Cell Line, Cancer Stem Cells

Ps-110: A 2-Wavelength Low Level Laser Irradiation Induces More *In Vitro* Cell Viability of Human Adipose Stem Cells Than Human Bone Marrow-Drived Stem Cells

Zare F*, Moradi A[1], Fallahnezhad S[2], Ghoreishi SK[3], Amini AA[1], Chien S[4], Bayat M[1]

1. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Statistics, University of Qom, Qom, Iran
4. Price Institute of Surgical Research, University of Louisville, and Noveratech LLC of Louisville, Louisville, Kentucky, USA
Email: fatemezare81@gmail.com

Background: The goal of the current experiment is to explore the influence of combined and/or single applications of red and near infrared (NIR) photobiomodulation (PBM) irradiation at different energy densities and sessions on cell viability, population doubling time (PDT), and apoptosis of *in vitro* cultures of human bone marrow-derived mesenchymal stem cells (hBMMSCs) and adipose-derived mesenchymal stem cells (hASCs).
Materials and Methods: Both *in vitro* hBMMSCs and hASCs were irradiated via 36 protocols that consisted of 4 different laser wavelengths (He-Ne laser, 630 nm, 810 nm, 630+810 nm); 3 different energy densities (0.6 J/cm2, 1.2 J/cm2, 2.4 J/cm2); and 3 different PBM sessions (1, 2, and 3).
Results: Irradiation with the 630 nm PBM significantly stimulated cellular viability and decreased PDT of both types of MSCs. The 630+810 nm NIR laser was statistically more effective and presented a synergistic effect compared to sole applications of the 630 nm and 810 lasers in terms of hBMMSC viability and PDT. However, there was significantly greater cell viability and PDT in hASCs irradiated 3 times with the 660+810 nm NIR laser at an energy density of 2.4 J/cm2 compared to the control group and hBMMSCs irradiated with the same protocol. PBM with 630 nm and the combined 630+810 nm wavelengths had a stimulatory effect on cell viability of *in vitro* hBMMSCs and hASCs.
Conclusion: However, the 630+810 nm PBM was statistically more effective. hASCs had a statistically better response to PBM in terms of cell viability and PDT than hBMMSCs .
Keywords: Human Bone Marrow-Derived Mesenchymal Stem Cells, Adipose-Derived Mesenchymal Stem Cells, Low Level Laser Irradiation, Cell Viability, Apoptosis

Authors Index

A

Abbasi F (Ps-71)
Abbasi J (Ps-48)
AbbasIs-Malati Z (Ps-8)
Abdijahed S (Ps-39)
Abedi M (Ps-56)
Abkari M (Ps-57)
Aboutaleb N (Ps-105)
Aboutalebi F (Ps-95)
Abtahi Froshani M (Ps-109)
Adibi Sedeh R (Ps-1)
Aflatoonian B (Ps-50, Ps-88)
Afshar AR (Ps-2)
Agata K (Ps-18)
Agata Kiyokazu A (Os-1)
Aghdami N (Ps-90)
Ahmadi M (Ps-3)
Ahmadian B (Ps-4)
Akbari B (Ps-90)
Akbari M (Ps-83)
Akhlaghpour A (Ps-5)
Akyash F (Ps-50, Ps-88)
Aliabedi B (Ps-6, Ps-82)
Aligholi H (Ps-57)
Alikhani M (Is-10, Ps-102)
Alini M (Is-18)
Alipanah H (Os-1)
Alizadeh Sh (Ps-6, Ps-82)
AlmasIs-turk S (Ps-21)
Amalia A (Os-3)
Amini AA (Ps-110)
Amini Sh (P-7, Ps-93)
Amiri F (Ps-8, Ps-9, Ps-10)
Amirpour N (Ps-93)
Ansari H (Is-12, Is-14)
Arefian E (Ps-79)
Arghiani N (Is-5)
Asadi H (Is-1, Ps-100)
Asakari MB (Ps-13)
Asfa S (Ps-11)
Asgari F (Ps-107)
Asgari Taei A (Ps-12)
Asghari MH (Ps-68)
Ashrafizadeh F (Ps-32)
Ashtari Kh (Ps-91)
Askari N (Ps-13)
Atlasi Y (Is-2, Is-3)
Avoj S (Ps-14)
Ayyari M (Ps-86)
Azari H (Ps-20, Ps-51)
Azimi Alamoti M (Ps-68)
Azmoodeh M (Ps-2)

B

Babashah S (Ps-24)
Bageban Eslaminejad MR (Ps-22, Ps-46, Ps-100)
Baghalishahi M (Ps-15)
Bagheri kakolaki F (Ps-16)
Bagheri R (Is-4)
Baghernezhad Shayan F (Ps-17)
Bagherzadeh R (Os-1, Ps-18)
Bahadori Z (Ps-5)
Baharvand H (Is-4, Is-10, Is-12, Is-14, Os-1, Ps-5, Ps-18, Ps-19, Ps-41, Ps-44, Ps-45, Ps-65, Ps-71, Ps-78, Ps-80, Ps-81, Ps-97, P-99, Ps-102)
Bahrami AR (Is-5)
Bahrami M (Is-5)
Barati M (Ps-19, Ps-78)
Barbosa M (Is-17)
Bargahi A (Ps-20)
Barmak AR (Ps-20)
Basiri M (Ps-41)
Basouli N (Ps-20)
Bassel-Duby R (Os-6)
Bayat M (Ps-110)
Becquart P (Is-17, Ps-21)
Bensidhoum M (Is-16)
Bidkhori HR (Is-5)
Bijarchian F (Ps-22)
Boisselier J (Is-17)
Boon R (Is-22, Is-23)
Bosch LVD (Is-24)
Braun T (Is-4, Ps-97)

C

Chavoshinejad R (Ps-77)
Chavoshinezhad S (Is-8, Ps-67)
Chien S (Ps-110)
Claes Ch (Is-24)
Crawford A (Is-6, Is-7, Ps-86)

D

Dadzadi M (Ps-23)
Daele JVD (Is-24)
Damme PhV (Is-24)
Danaeinezhad Sh (Ps-24)
Daneshi S (Ps-51)
Dargahi L (Is-8, Ps-12, Ps-55, Ps-67)
Darya Gh (Ps-25)
Dastpak M (Is-5)
De Smedt J (Is-22)
De Strooper B (Is-24)
Deboeck J (Is-22, Is-23)
Decambron A (Is-16)
Dehghan AR (Ps-26)

Delirezh N (Ps-47, Ps-58, Ps-109)
Denoeud C (Is-17)
D'Este M (Is-9, Is-18)
Deviatiiarov R (Ps-18)
Diallo A (Is-17)
Dinarvand R (Ps-68)
Dirami T (Os-6)
Dormiani K (Os-4, Ps-52, Ps-95)
E
Ebad M (Ps-27)
Ebrahimi M (Ps-6, Ps-19, Ps-68, Ps-82)
Ebrahimi S (Ps-64)
Ebrahimi Warkiani M (Ps-91)
EbrahimIs-Barough S (Ps-66)
Ebrahimpour-Komleh H (Ps-77)
Eftekhar M (Ps-50)
Eftekhar-Vaghefi SH (Ps-15)
Eglin D (Is-18)
Emadi R (Ps-76)
Emadi RA (Ps-96)
Eremin II (Is-13)
Eslami B (Ps-77)
Esmaeilzadeh N (Ps-91)
F
Faghih H (Ps-24, Ps-28)
Fakhimahmadi A (Ps-42)
Fakhr Taha M (Ps-28)
Fallahi H (Ps-29, Ps-40, Ps-74)
Fallahnezhad S (Ps-110)
Falsafi N (Ps-29)
Farahi S (Ps-30)
Farahmandfar M (Ps-12)
Farahzadi R (Ps-31, Ps-35, Ps-36)
Farasatkia A (Ps-32)
Farasatpour Z (Ps-33)
Farashahi Yazd E (Ps-50, Ps-88)
Farhadi A (Ps-34)
Faried A (Os-7)
Farifteh F (Ps-30)
Farivar Sh (Ps-39)
Farrar Z (Ps-2)
Farshchian M (Is-5)
Farzaneh Z (Ps-59, Ps-80)
Fathi E (Ps-31, Ps-35, Ps-36)
Febriansah R (Ps-37)
FozonIs-Moghaddam B (Is-5)
Furtado R (Is-24)
G
Garcia Leon J (Is-24)
Ghadami SA (Ps-5)
Ghaderi Gandomani M (Ps-38)
Ghaedi K (Ps-11)
Ghafouri S (Ps-39)
Ghahremani MH (Ps-94)
Ghamari N (Ps-40, Ps-74)
Ghanian MH (Ps-44)
Ghasemi H (Ps-64)
Ghasemi Hamidabadi H (Ps-67)
Gheisari Y (Ps-1, Ps-56)
Ghezel Ayagh Z (P-99)
Ghobeh M (Ps-87)
Ghodsi A (Ps-19)
Ghoreishi SK (Ps-110)
Ghosh S (Is-22)
Girsang E (Os-7)
Givzad N (Ps-55)
Golzadeh J (Ps-88)
Gorkun AA (Is-13)
Grebenik EA (Is-21)
Grijpma DW (Is-18)
Grunsven LV (Is-22)
Gusev O (Os-1, Ps-18)
H
Habibi Roudkenar M (Ps-8)
Hadad P (Ps-3)
Haddadi M (Ps-41)
Haeri A (Ps-59)
Haghi A (Ps-42, Ps-61)
Haghjoo Javanmard Sh (Ps-96)
Hajimiri MH (Ps-68)
Hajinasrollah M (Ps-65)
Hajizadeh F (Ps-88)
Hajizadeh-Saffar E (Ps-41, Ps-65, Ps-71)
Hanaee-Ahvaz H (Ps-49)
Hasani Fard AH (Ps-43)
Hasanzade H (Is-5)
Hashemi AR (Ps-21)
Hashemi F (Ps-25)
Hassani SN (Is-10, Ps-5, Ps-19, Ps-71, Ps-78, Ps-99)
Hassanzadeh GH (Ps-57, Ps-83)
Hassanzadeh GhR (Ps-12)
Haumaitre C (Os-6)
Hayati Roodbari N (Ps-49)
Hedayati Asl AA (Ps-6, Ps-82)
Helsen N (Is-22)
Heravi M (Ps-67)
Heriady Y (Os-7)
Heydari H (Ps-25)
Heydari Z (Ps-44)
Hill CH (Os-5)
Homayouni Moghadam F (Ps-52)
Hosseini Aghdaei F (Is-12)
Hosseini F (Ps-86)
Hosseini M (Ps-67)
Hosseini Mazinani FS (Ps-45)
Hosseini S (Ps-46)
Hosseini Salekdeh GH (Is-10, Is-14, Ps-102)
Hosseinpoor H (Ps-49)

Hosseinzadeh M (Ps-46)
Hu W (Is-22)

I

Izadi ZH (Is-11)
Izadpanah AH (Ps-47)

J

Jabbari Fakhr M (Ps-48, Ps-69)
Jahanbazi Jahan-Abad A (Ps-87)
Jalali H (Ps-43)
Jalili A (Ps-63)
Jamshidi M (Ps-68)
Janzamin E (Ps-19, Ps-78)
Jasaputra D (Os-7)
Javadzade H (Ps-21)
Javanmardi S (Ps-35)
Javanshir Sh (Ps-72)
Javeri A (Ps-28)
Jenab Y (Ps-65)

K

Kabiri M (Ps-49)
Kadivar M (Ps-12)
Kamal A (Ps-3)
Kamkar L (Ps-3)
Karamali F (Ps-14, Ps-33)
KarbalaeiMahdi A (Ps-63)
Karima S (Ps-87)
Karimi A (Ps-14)
Karimi Tar H (Ps-67)
Karimzadeh F (Ps-62)
Kashef-Saberi MS (Ps-49)
Kay M (Is-12)
Kazem Arki M (Ps-49)
Kazemnejad S (Ps-85)
Keller A (Ps-3)
Kermani F (Os-2)
Kermanpour A (Ps-104)
Keshavarz Sh (Ps-50)
Keshtkar MR (Ps-20)
Keshvari MA (Ps-51)
Khademi F (Ps-98)
Khamisipour GhR (Ps-21)
Khani F (Is-10)
Kharaziha M (Ps-32, Ps- 62, Ps-76, Ps-96, Ps-104)
Khataminejad M (Ps-20)
Khayam Abed R (Ps-95)
Khayamabed R (Ps-52)
Khazaei F (Ps-53, Ps-54)
Khazaei M (Ps-53, Ps-54, Ps-60)
Khazaei MR (Ps-60)
Khodabakhsh P (Ps-55)
Khoddami V (Ps-97)
Khoshkhoo A (Ps-21)
Kiani T (Ps-5, P-99)
King O (Os-2)
Kiyanpour F (Ps-56)
Kordi Tamandani DM (Ps-38)
Koruji M (Ps-92, Ps-91, Ps-107)
Kosheleva NV (Is-13)
Kuhestani B (Ps-106)
Kumar M (Is-22, Is-23)
Kusuma H (Os-3)

L

Larreta-Garde V (Is-17)
Lashkari M (Ps-3)
Lazhko AE (Is-21)
Lecuelle B (Is-16)
Lefebvre E (Is-17)
Lister I (Os-7)
Logeart-Avramoglou D (Is-16)
Lotherington L (Ps-86)
Lubis WA (Ps-37)

M

Mahdavipour M (Ps-57, Ps-83)
Mahmodlou R (Ps-47)
Mahmoudian AR (Ps-108)
Mahmoudpour M (Ps-20)
Mahmudpour M (Ps-51)
Maleki Sh (Ps-58)
Manassero M (Is-16)
Marinesco S (Is-17)
Marlina M (Os-3)
Masaeli E (Ps-89)
Matin MM (Is-5)
Mehrzad S (Is-5)
Meiller A (Is-17)
Meuleman Ph (Is-23)
Meyfour A (Is-10, Is-14)
Minkina E (Ps-18)
Mirahmadi M (Is-5)
Miri Lavasani Z (Ps-59)
Miri MR (Ps-51)
Mirmohammadi R (Ps-50)
Mirzae F (Ps-51)
Mirzaei Sh (Ps-2)
Mirzapur P (Ps-60)
Mmahdavi B (Os-4)
Moayedi Rad M (Ps-25)
Moein Sh (Ps-1)
Mohajer Ansari J (Ps-91)
Mohammad M (Ps-6, Ps-42 , Ps-82)
Mohammad Soltani B (Is-12)
Mohammadi Kian M (Ps-61)
Mohammadi M (Ps-50)
Mohammadi S (Ps-42, Ps-61)
Mohebbi GhH (Ps-20)
Mohseni Kouchesfahani H (Ps-43)
Moini A (Ps-83)
Mokhber Dezfouli MR (Ps-48, Ps-69)
Mokhtari H (Ps-104)

Mokhtari HR (Ps-62)
Mola Mohammadi S (P-99)
Molaei S (Ps-9, Ps-10)
Molamohamadi S (Ps-5 , Ps-81, Ps-102)
Molamohammadi S ()
Mollaei HR (Ps-15)
Mollamohammadi S (IS-10)
Momeni M (Ps-68)
Montgomery R (Os-6)
Moosavi MA (Ps-42)
Moradi A (Ps-110)
Moradi SH (Is-4, P-99, Ps-97)
Moridi K (Ps-63)
Moshtaghioun SM (Ps-50)
Mousavi SE (Ps-23)
Mousavi SH (Ps-6, Ps-82)
Mousavinejad M (Os-5)
Movahed M (Os-6)
Movahedin M (Ps-107)
Mowla SJ (Is-15, Ps-107)
Moya A (Is-16)
Muhaddesi M (Ps-21)
Murray P (Os-5)
N
Nabipour I (Ps-20, Ps-51)
Naderi Meshkin H (Ps-72, Ps-73)
Nafian S (Is-10)
Nami A (Id-23)
Narimanpour Z (Ps-64)
Naseroleslami M (Ps-105)
Nasoohi S (Ps-12)
Nasr Esfahani MH (Os-4, Ps-11, Ps-14, Ps-33, Ps-52, Ps-95)
Navabi R (Ps-65)
Nazm Bojnordi M (Ps-64, Ps-66, Ps-67)
Negahdari B (Ps-41, Ps-65)
Nejabat GR (Ps-98)
NematollahIs-Mahani SN (Ps-15)
Neyrink K (IS-23)
Neyts J (IS-21)
Nikbakht M (Ps-42, Ps-61)
Niyazi M (Ps-67)
Noori A (Ps-69)
Noory P (Ps-70)
Noruzinia M (Ps-107)
Nowroozi MR (Ps-107)
O
Olson E (Os-6)
P
Pahlavan S (Is-14)
Pakzad M (Is-4, Ps-71)
Paquet J (Is-16)
Parham A (Ps-72, Ps-73)
Parivar K (Ps-49)
Parvaneh Tafreshi A (Ps-17, Ps-79)
Pauthe E (Is-16)
Pazhouhi M (Ps-53, Ps-54)
Petite H (Is-16, Is-15)
Petta D (Is-18)
Peymani M (Ps-11)
Piryaei A (Ps-15, Ps-59, Ps-80)
Potier E (Is-17)
Pournasr B (Ps-80)
Pulin AA (Is-13)
Q
Quilichini E (Os-6)
R
Rabbani Sh (Ps-65)
Radak M (Ps-40, Ps-74)
Rafatpanah H (Is-5)
Rafiee Z (Ps-75)
Rahmadian R (Os-3)
Rahmati M (Ps-42)
Rajabi Dehnavi N (Ps-76)
Rajabi S (Ps-90, Ps-103)
Ramezani M (Is-19)
Rassouli H (Ps-19)
Razi M (Ps-108)
Rezaei N (Os-4, (Ps-52)
Rezaei Z (Ps-77)
Rizal R (Os-3, Os-7)
Roshandel E (Ps-106)
Roskams T (Is-23)
Ross J (Is-23)
S
Sabahi Moosavi N (Ps-19, Ps-78)
Saburina IN (Is-13)
Sadeghi E (Ps-67)
Sadeghi H (Ps-3)
Sadeghi Y (Ps-15)
Sadeghian Chaleshtori S (Ps-69)
Sahebdel F (Ps-79)
Sahebghadam Lotfi A (Ps-38)
Saheli M (Ps-80)
Saki Gh (Ps-75)
Salari AA (Ps-87)
Salehi H (Ps-93)
Salehi M (Ps-30)
Salehi S (Ps-32)
Salemi M (Ps-42, Ps-61)
Salimi A (Ps-63)
Salimi E (Ps-53)
Salmani F (Ps-87)
Samadian A (Ps-5, Ps-81, P-99)
Samieyan Dehkordi S (Ps-6, Ps-82)
Sanati MH (Ps-45)
Sankanian Gh (Ps-106)
Saremi J (Ps-64, Ps-66)
Satarian L (Ps-45)

Satrio W (Os-3)
Savoj S (Ps-33)
Seifali E (Ps-57, Ps-83)
Sepahi MM (Ps-84, Ps-85)
Sepantafar MM (Ps-80)
Shaban Malekshah D (Ps-48)
Shabani R (Ps-91, Ps-92)
Shahbazi N (Ps-86)
Shahpasand K (Ps-16, Ps-23, Ps-34, Ps-87)
Shahpasand Sh (Ps-87)
Shahriary Sh (Ps-88)
Shams A (Ps-89, Ps-91, Ps-92)
Shams Z (Ps-90)
Shamsara M (Ps-28)
Shariati Najafabadi S (Ps-93)
Sharif E (Is-19)
Sharif Mashadi S (Ps-94)
Sharif Zarchi A (Os-1, Ps-3, Ps-18)
Shayan Asl N (Ps-68)
Shayesteh Pour B (Ps-3)
Shekari F (Ps-57)
Shokri B (Ps-39)
Shokrolahi F (Ps-98)
Siavashi S (Ps-95)
Sodeifi N (Ps-68)
Soleimani L (Ps-25)
Soleimani M (Ps-94)
Soltanalinejad F (Ps-108)
Soltani S (Ps-76, Ps-96)
Sonda S (Os-6)
Soori S (Ps-97)
Sørum H (Ps-86)
Stedman A (Os-6)

T

Tabatabaei SA (Ps-98)
Taee A (Is-10)
Taei A (Ps-5, P-99)
Taghiyar L (Ps-22, Ps-100)
Tahajjodi SS (Ps-88)
Tahamtani Y (Ps-34, Ps-86)
Tahouri M (Ps-101)
Taleahmad F (Ps-102)
Taleahmad S (Is-10, Ps-102, Ps-81)
Tamadon A (Ps-2, Ps-20, Ps-21, Ps-51)
Tamimi M (Ps-103)
Tanbakoei S (Ps-91)
Tavakoli F (Ps-106)
Tavakoli Sh (Ps-62, Ps-104)
Taylor A (Os-5)
Tekiyeh Maroof N (Ps-105)
Terracciano C (Os-2)
Timashev PS (Is-21)
Topraksihar B (Is-22, Is-23)
Totonchi M (Ps-3, Ps-77)
Tricot T (Is-22, Is-23)

U

Ubaydillah M (Os-3)

V

Vahdani N (Ps-106)
Vahidi B (Ps-4, Ps-27)
Verfaillie C (Is-22, Is-23, Is-24)
Viateau V (Is-16)
Vietor I (Ps-35, Ps-36)
Vojoudi E (Ps-66)
Vosough M (Ps-44, Ps-59, Ps-80)

W

Wahyu W (Os-3)
Wibowo S (Os-7)
Widodo W (Os-3, Os-7)
Widowati W (Os-7)

Y

Yakhkeshi S (Ps-5)
Yanti Ni Luh (Os-3)
Younesi Soltani F (Ps-73)

Z

Zabolian AH (Ps-25,
Zadpoor AA (Is-25, Is-26)
Zahiri M (Ps-107)
Zahmatkesh E (Ps-59)
Zaker FS (Ps-108)
Zand S (Ps-58, Ps-109)
Zare F (Ps-110)
Zare N (Ps-2)
Zarkesh L (Ps-44)
Zeynali B (Ps-17, Ps-79)
Zibaii MI (Ps-67)
Zolfaghari AP (Ps-74)
Zurina IM (Is-13)