ROYAN

International Hybrid Twin Congress

Reproductive Biomedicine & Stem Cells

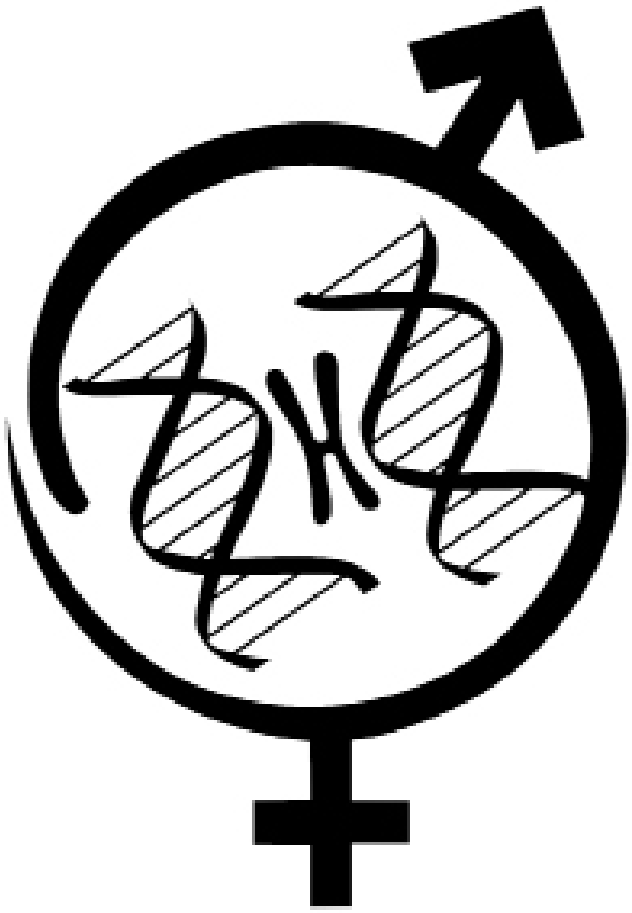
7 - 9 September, 2022 Tehran - Iran

18th Congress on Stem Cell Biology & Technology

Abstracts of

Royan International Hybrid Twin Congress

18th Congress on Stem Cell Biology and Technology
7-9 September 2022



Royan Institute

Cell Science Research Center

Abstracts of the 18th Congress on Stem Cell Biology and Technology (2022)

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Congress Chairperson

Hamed Daemi

On behalf of the organizing committee, it is my great pleasure to welcome you to the 18th International Hybrid Congress on Stem Cell Biology and Technology (ICSCBT), 7-9 September 2022.

The ICSCBT is an international annual gathering in stem cells research and regenerative medicine covering different aspects of basic and translational contents to feature a global line-up of scientists and clinicians. Scientific and friendly discussion on our impediments and innovation, and sharing our recent cutting-edge research in the field of stem cell are the main aims of ICSCBT.

Royan institute was established in 1991 by the late Dr. Kazemi Ashtiani. As a pioneer institute, Royan Institute for Stem Cell Biology and Technology (RI-SCBT) embraced stem cells basic and translational studies, developmental biology and regenerative medicine.

We kindly invite you to join us in Tehran for a motivating and enjoyable ICSCBT 2022 to learn, share and network.

Kind regards
Hamed Daemi, Ph.D
Chairperson of 18th Royan International Congress
on Stem Cell Biology & Technology

Invited Speakers

Is-1: Functional Two-Dimensional Polyols for Biomedical Applications

Adeli M

Lorestan University, Department of Chemistry, Khorramabad, Iran
Sharif University of Technology, Department of Chemistry, Tehran, Iran
Email: mohadeli@yahoo.com

Two-dimensional nanomaterials (2DNs) have been in the forefront of cutting-edge interdisciplinary research fields, since exploring graphene at 2004. They have shown great potential for a wide range of biomedical applications including drug delivery, virus interactions and tissue engineering. The synthesis methods of 2DNs can be classified in "top-down" strategies including the physical, mechanical and chemical exfoliation of 3D layered structures and "bottom-up" approaches such as chemical vapor deposition and wet-chemistry protocols. Owing to their multivalent interactions at biointerfaces, the biological behaviours and therapeutic efficiency of 2DNs is dominated by their functionality. Therefore, functionalization of 2DNs is a crucial step to formulate them in different biomedical applications. We have been working on this topic for a decade to explore different parameters affecting multivalent interactions at these nanobiointerfaces and deep understanding of the mechanism of these interactions. We explain, how a change in the functionality of 2DNs can be manifested in the physicochemical properties of 2DNs which in turn has a huge effect on their biological behaviours and differentiation in different categories in terms of therapeutic effects, toxicity, inter- and intracellular localizations.

Keywords: Biointeractions, Two-Dimensional Nanomaterials, Two-Dimensional Polymers, Virus Interactions, Wound Healing

Is-2: Multilayer Nano-Microstructures for Smart Drug Delivery

Ajalloueian F

IDUN Center of Excellence, Department of Health Technology, Technical University of Denmark, Denmark
Email: faaj@dtu.dk

Nano and Micro-based delivery systems are representing rapidly developing science where materials in the nano and/or micro scale range are employed to deliver therapeutic agents to specific targeted sites in a controlled manner. Right selection of the biomaterials and the applied fabrication technique depending on the delivery route of interest (oral, dermal, nasal, etc.) are the key factors to guarantee an efficient, noninvasive and convenient drug delivery.

An example is local chemotherapy with the advantage of providing a high concentration of drug directly into the tumor site and thereby decreasing the side effects associated with drug cytotoxicity. In one of our studies, we fabricated an electrospun composite patch including a co-drug-loaded graphene oxide-based nanocarrier for local breast cancer application and demonstrated a synergistic cytotoxicity effect of the applied drugs.

Oral drug delivery is another attractive method among various delivery routes. Oral dosage forms are still the gold standard for the treatment and management of chronic and debilitating diseases. Compared to conventional single-unit dosage forms, micro and nanoparticles have gained increasing interest for development of novel gastrointestinal drug delivery systems. Whilst some superiorities are reported for nanoparticles including larger surface-area-to-volume ratio, more uniform distribution and higher cellular uptakes, microparticles also benefit from several advantages such as enhanced peptide stability, improved protection against enzymatic degradation, and facilitated oral absorption. Such characteristics raise the need to make use of both nano and microparticulate formulations for maximum benefit. To this end, we have designed, fabricated and characterized novel Multilayer Nanofibrous Microparticle systems for smart oral drug and cell delivery applications. The multilayer construct provides us with the potential to load various drugs or bioactive agents in different layers where other factors such as hydrophilicity/hydrophobicity of the applied biopolymers, the thickness of each layer and the compression pressure during cutting can alter the delivery site or change the release trend.

Is-3: Intercellular Communication by Exosome-Derived miRNAs within The Tumor Microenvironment in Breast Cancer

Babashah S

Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: babashah@modares.ac.ir

The process of tumor formation and progression is influenced by two factors, namely genetic/epigenetic changes in the tumor cells and the rearrangement of the components of the tumor microenvironment (TME) through mutual and dynamic crosstalk. TME consisting of distinct cell types including stromal cells and immune cells has recently emerged as a pivotal player in tumor development and progression. Over the last decade, researchers have demonstrated that mesenchymal stromal/stem cells (MSCs) can also migrate to the tumor microenvironment. Even though there has been an intense interest in the role of MSCs in cancer progression, the relationship between MSCs and tumor cells remains open to debate. Several studies have suggested that MSCs contribute to tumor progression and metastasis, whereas other reports have shown that MSCs suppress tumor growth. MSCs have been shown to be involved in the formation and modulation of tumor stroma and in interacting with tumor cells, partly through their secretome. Exosomes are nano-sized intraluminal multivesicular bodies secreted by most types of cells and have been found to mediate intercellular communication through the transfer of genetic information via coding and non-coding RNAs to recipient cells. Since exosomes are considered as protective and enriched sources of shuttle microRNAs (miRNAs), we hypothesized that exosomal transfer of miRNAs from MSCs may affect tumor cell behavior, particularly angiogenesis. However, molecular mechanisms underlying interactions between MSCs and tumor cells in breast cancer progression need to be thoroughly elucidated, which may provide attractive targets for making promising novel strategies for breast cancer therapy.

Is-4: GMP CAR T Cell Production: Practical Aspects and Prospects

Basiri M

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: m.basiri@royan-rc.ac.ir

Is-5: Clonal Competition in The Normal Oesophageal Epithelium During Early Carcinogenesis

Colom B

Wellcome Sanger Institute, Pre-Cancer Group, Wellcome Genome Campus, Hinxton, Cambridge, UK
Email: bc5@sanger.ac.uk

Human epithelial tissues accumulate cancer-driver mutations with age, yet tumour formation remains rare. The positive selection of these mutations argues they alter the behaviour and fitness of proliferating cells. Hence, normal adult tissues become a patchwork of mutant clones competing for space and survival, with the fittest clones expanding by eliminating their less-competitive neighbours. However, little is known about how such dynamic competition in normal epithelia influences early tumorigenesis. To answer this question, we have developed an *in vivo* mouse model that recapitulates the mutational landscape of the normal human oesophageal epithelium. We have used transgenic mouse models, quantitative lineage tracing, ultra-deep DNA sequencing, whole-tissue confocal imaging and mathematical modelling to resolve the dynamics of mutant clones in this tissue, and their role in the development of emerging tumours. Our results show that mutant clone dynamics in the normal oesophageal epithelium are defined by the relative fitness of clones competing for survival within the tissue confined space. In this environment, clones carrying particular mutations prevent early tumorigenesis by keeping in check more malignant clones, and by directly outcompeting and eliminating emerging micro-tumours. Importantly, manipulating the normal epithelium mutational landscape influences early tumour formation, an approach that may lead to the development of novel preventive strategies against cancer. We conclude that mutant clones in normal epithelium have an unexpected anti-tumorigenic role in purging early tumors through cell competition, thereby preserving tissue integrity.

Is-6: Mechanism of Extracellular Vesicles Derived from MSCs in Implantation: A Meta-Transcriptomic Analysis

Dalirfardouei R[1, 2]

1. Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2. Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
Email: r.dalir@umsha.ac.ir

Background: A growing body of evidence has shown that extracellular vesicles (EV) can be efficient on 3 steps of embryo implantation however there is a significant unmet need to understand comprehensively how EVs derived from mesenchymal stem cell (MSC) are involved in the embryo implantation. The objective of the research is to gain a better insight into EVs therapeutic mechanisms.
Materials and Methods: MicroRNA profiles of extracellular vesicles from MSC were achieved by searching in literature and GEO databases. The studies which applied well-characterized MSCs and their EVs based on ISCT and ISEV guidelines, respectively, were selected for further analysis.
To provide a functional biological network with target genes of common miRNAs, the StRING software was applied and then visualized using Cytoscape 3.7.1. To analyze the network and find significant biological clusters, the Molecular Complex Detection (MCODE) Cytoscape plugin was used. Functional enrichment and gene ontology (GO) analysis were performed using DAVID Bioinformatic Resources.
Results: According to the Venn diagram, only a small portion of miRNAs are overlapped among protein lists. These shared common miRNAs were associated with signaling pathways such as "IL-4, IL-13 signaling pathway", "NOTCH signaling", "PIP3 activates AKT signaling" as well as several other pathways related to reproduction, supporting that EVs from MSC was expected to positively impact on embryo development.
Conclusion: The central themes of biological process of hub genes such as the "in utero embryonic development", "embryo implantation", and "prostate gland developement" confirmed the conclusion that MSC derived EVs play a key role in implantation and treatment of reproductive diseases.
Keywords: Bioinformatics Analysis, Extracellular Vesicles, Implantation, Mesenchymal Stem Cells, MicroRNA, Reproductive Disease, Transcriptomics

Is-7: Novel collagen nanomaterials for stem cell-based Tissue Engineering

Davaran S

Department of Medical Nanotechnology, Faculty of Advanced Medical Science, Tabriz University of Medical Science, Tabriz, Iran
Email: davaran@tbzmed.ac.ir

Is-8: Stars in The Galaxy: Microparticles as Toolkits for Engineering Cell Spheroids

Ghanian MH

Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: mh.ghanian@royan-rc.ac.ir

Cell spheroids are 3D aggregates of one or more type of the cells that are gaining high interest because of their superior functionality caused by 3D cell-cell and cell-ECM interactions and their potential for upscaling in suspension cultures. Cell-sized microparticles are modulable engineering toolkits that can be used to locally control the biochemical and biophysical milieu of the microenvironment within 3D multicellular aggregates. Since recent decade, we have developed microparticles of different synthetic or natural materials with adjusted physicochemical properties and incorporated them within the stem cell spheroids or organoids to regulate stem cell differentiation or maintain functionality of the mature cells.

Is-9: Challenges and Steps in the Development of Advanced Therapy Medicinal Products

Hajizadeh-Saffar E

Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: en.hajizadeh@royan-rc.ac.ir

The introduction of advanced therapy medicinal products (ATMPs) to the global market has been revolutionizing the pharmaceutical industry, brings new ways for treating various incurable diseases. These products are developed in academic and/or hospital laboratories and need to be adjusted in regards to the standards of GMP manufacturing and transferred to specialized Contract Manufacturing Organization or certain GMP facilities. The complexity of ATMPs brings various challenges, containing the source of starting and raw material, the technical know-how required to produce and related quality control of the products in the respect of regulatory and GMP compliance. Clinical development of ATMP encounters specific challenges due to the nature of the product and the limited availability of non-clinical data. Validation of manufacturing and testing of these products particularly with regard to identity, purity, safety and potency has great importance. The restricted accessible appropriate standards and reference material along with an inadequacy in certain guidelines are the other challenges.
To translate a laboratory scale product to an industrial scale we need a quality management system that includes Quality Assurance (QA) and Quality Control (QC) which should be applied in all procedures in a GMP facility. The establishment and maintenance of a quality management system could assure the long-term sustainability of the product. Furthermore, quality systems are necessary for the delivery of high-quality products to the end-user.
Inspection is the process of measuring, examining, and testing to check all characteristics of a product or service and the comparison of these with specified requirements to determine conformity. Products, processes, and various other results can be inspected to make sure that the object coming off a production line, or the service being provided, is correct and meets specifications.

Is-10: Building Implantable Human Liver Tissue from Pluripotent Stem Cells

Hay D

Centre for Regenerative Medicine, Institute for Regeneration and Repair, The University of Edinburgh, Edinburgh, UK
Email: davehay@talktalk.net

Liver disease represents an increasing cause of global morbidity and mortality. Currently, liver transplant is the only treatment curative for end stage liver disease. Donor organs cannot meet the demand and therefore, scalable treatments and new disease models are required to improve clinical intervention.
Pluripotent stem cells represent a renewable source of human tissue. Recent advances in three-dimensional cell culture has provided the field with more complex systems that better mimic liver physiology and function. Despite these improvements, current cell-based models are highly variable in performance and expensive to manufacture at scale. This is due, in part, to the use of poorly defined or cross-species materials within the process, severely affecting technology translation. To address this issue, we have developed an automated and economical platform to produce liver tissue at scale for modelling disease and small molecule screening. Stem cell derived liver spheres were formed by combining hepatic progenitors with endothelial cells and stellate cells, in the ratios found within the liver. The resulting tissue permitted the study of human liver biology 'in the dish 'and could be scaled to levels that may prove useful to supporting failing liver function in humans.

Is-11: Electrically Conductive Ccaffolds for Tissue Engineering: Advantages, Challenges, and Perspectives

Jaymand M

Nano Drug Delivery Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: mehdi.jaymand@kums.ac.ir

Objective: Tissue and organ failure resulted from injuries, diseases or another type of damages is one of the most important health problems. In this context, the most common clinical treatment approaches are surgical repair, artificial prostheses, drug therapy, mechanical devices, and transplantation. Nevertheless, the regeneration or healing of damaged and failed tissue/organ are not satisfactory in all cases. Tissue engineering (TE) has been developed as a powerful alternative for the above-mentioned treatments. TE is a multi-disciplinary science that combines materials engineering and molecular biology to develop biological substitutes consisting signaling molecules, living cells, and scaffolds that can be used for healing or regeneration of damaged or failed tissues/organs.
Amongst, the scaffold provide physicochemical cues for living cells and has an important role in their missions, including adherence, proliferation, and differentiation. In addition, electrical conductivity of scaffold has inherent role in some biological functions (e.g., cell adhesion, cell migration, cell differentiation, and DNA synthesis). These biomaterials are divided into two main categories, including conductive polymers-based scaffolds and conductive nanomaterials-based scaffolds. The most exploited nanomaterials are carbon-based nano-materials (e.g., carbon nanotubes and graphene) and gold nanoparticles. Nevertheless, the main problem of this strategy are non-biodegradability and inflammatory reactions of fillers *in vivo*. Conducting polymers (CPs) such as polyaniline (PANI), polypyrrole (PPy), polythiophene (PTh), and their derivatives can be considered as potential candidates for the fabrication of conductive scaffolding biomaterials owing to their superior physicochemical properties as well as acceptable *in vitro* and *in vivo* cyto- and bio-compatibilities in purified form. Nevertheless, elongated *in vivo* degradation time of these polymers may result in inflammation and requirement of surgical removal. To overcome such problem, modification of CPs with natural and synthetic biodegradable and biocompatible polymers through grafting or blending processes has suggested as efficient approach.
Materials and methods: As above discussed facts, in recent years, we have focused on design and development of electrically conductive scaffolding polymeric biomaterials through modification of PANI, PPy and PTh with natural and synthetic biodegradable and biocompatible polymers. In this regard, some electrically conductive scaffolds with these biomaterials in electrospun nanofibers and hydrogel forms were fabricated in our laboratory. The physicochemical (e.g., morphology, hydrophilicity, swelling, conductivity, and electroactivity) as well as biological (biodegradation, cyto- and hemo-compatibility, and improve the cells adhesion and proliferation) properties of the fabricated scaffolds were investigated.
Results: It was found that the developed electrically conductive

scaffolds through the grafting of PANI, PPy and PTh onto aliphatic polyester, gelatin, chitosan, polydopamine, polylactide, poly(2-hydroxy ethylmathacrylate) and poly(ethylene glycol) exhibited acceptable electrical conductivities and electeroactivities. In most cases, the fabricated scaffolds showed excellent *in vitro* biodegradation rate that assessed by both gravimetric approach and SEM imaging. In comparison with polystyrene plate (as control) the developed scaffolds displayed better cells adhesion and proliferation performance. The hemolysis assay approved the hemocompatibilities of the developed scaffolding biomaterials, and in the most cases the hemolysis rate were less than 3% even at relatively high concentration.
Conclusion: Physicochemical as well as biological analysis approved the potential of developed electrically conductive scaffolds for various TE, especially in the case of electrically excitable cells (e.g., fibroblast, osteoblast, and neural) owing to importance effect of electrical conductivity on cell's adhesion, migration and differentiation, as well as DNA synthesis and protein secretion. However, it is extremely important to optimize the composition of conductive scaffolding biomaterials based on the cytotoxicity threshold.
Keywords: Conductive polymers, Modification, Electrically conductive scaffolds, Tissue engineering

Is-12: Biocompatibility Issues for The Tissue Engineered Products for Commercialization

Khang G

Department of PolymerNano Sci & Tech, Jeonbuk National University, Jeonju, South Korea
Email: gskhang@jbnu.ac.kr

Around 1992 as 20 years ago, Advance Tissue Science Co (USA), now merged to Smith & Nephew Co., USA, had been submitted to approve to USA FDA for first cartilage TEMPs as autologous chondrocyte/polyglycolic acid (PGA) nonwoven scaffold. At that time, no one had doubted to approve cartilage TEMPs since PGA was already approved by FDA in human clinical trial and chondrocyte was used autologous primary cell. At last, this product has been still retard up to approve FDA. Main reason might be in terms of safety. Implanted TEMPs have been reported to induce sequential events of immunologic reactions in response to injury caused by implantation procedures and result in acute inflammation marked by a dense infiltration of inflammation-mediating cells at the materials-tissue interface. Prolonged irritations provoked by implanted biomaterials advance acute inflammation into chronic adverse tissue response characterized by the accumulation of dense fibrotic tissue encapsulating the implants.
In this lecture, we will discuss 1. recent advances for the commercialization trends for the tissue engineered products (TEMPS) including regenerative medicinal products, 2. scaffolds in terms of biocompatibility and safety issue, 3. smart scaffold for the application of clinical trial including improved biocompatibility and the reduction of host response, and 4. biocompatibility issue for the natural and synthetic polymers.

Is-13: mRNA; A Transformative Technology for Disease Prevention & Treatment

Khoddami V

Pediatric Cell and Gene Therapy Research Center, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
Email: v.khoddami@renap.ir

"The brand new mRNA technology has successfully introduced its first important and effective products; the COVID-19 mRNA vaccines from Pfizer and Moderna in 2020. These two vaccines and many more vaccines and other mRNA-based products are expected to appear in the drug market from these two companies and many more startups world-wide within the next few years. It seems that mRNA technology is about to revolutionize the biopharmaceutical industry by introducing a wide variety of products for disease control and treatment. Here, in Iran we have developed COReNAPCIN® the first mRNA-based vaccine candidate. We are also working on the other aspects of mRNA technology applicable in gene- and immuno-therapy as well as regenerative medicine. In this talk I will present the recent advances regarding establishment of mRNA technology and its supply chain in Iran, for sustainable development of domestic prophylactic vaccines and drug products for the benefit of the society.

Is-14: Janus Nanoparticles: from Design to Bioapplication

Khoee S

Polymer Laboratory, School of Chemistry, College of Science, University of Tehran, Tehran, Iran
Email: khoee@khayam.ut.ac.ir

Janus particles, which are named after the two-faced Roman god Janus, have two distinct sides with different surface features, structures, and compositions. These two faces have various physical or chemical characteristics, which endow their unique properties. This anisotropic structure has excellent performance in various fields, especially in biomedical applications. Herein, comprehensively detailing fabrication strategies will be introduced based on their materials. The JNPs are formed from three types of materials: 1) polymeric composites, 2) inorganic composites, and 3) hybrid polymeric/inorganic JNPs composites. Finally, some of their biomedical applications will be affiliated. As a biomedical device, Janus particles offer opportunities to incorporate therapeutics, imaging, or sensing modalities in independent compartments of a single particle in a spatially controlled manner. This may result in synergistic actions of combined therapies and multi-level targeting not possible in isotropic systems.

Is-15: Cell Size Determines Stem Cell Potential During Aging

Lengefeld J

Institute of Biotechnology, University of Helsinki; Department of Biosciences and Nutrition, Karolinska Institutet
Email: jette.lengefeld@helsinki.fi

Stem cells are remarkably small. Whether small size is important for stem cell function is unknown. We find that hematopoietic stem cells (HSCs) enlarge under conditions known to decrease stem cell function. This decreased fitness of large HSCs is due to reduced proliferation and was accompanied by altered metabolism. Preventing HSC enlargement or reducing large HSCs in size averts the loss of stem cell potential under conditions causing stem cell exhaustion. Last, we show that murine and human HSCs enlarge during aging. Preventing this

age-dependent enlargement improves HSC function. We conclude that small cell size is important for stem cell function *in vivo* and propose that stem cell enlargement contributes to their functional decline during aging.

Is-16: The Increasing of Cell-Based Immunotherapy Products Application in Cancers

Madani H[1], Hamidieh AA[2*]

1. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Pediatric Cell and Gene Therapy Research Center, Gene, Cell and Tissue Research Institute, Tehran University of Medical Sciences, Tehran, Iran
Email: aahamidieh@tums.ac.ir

The era of Regenerative Medicine as future medicine is upon us. Thousands of Ideas to market have demonstrated profound, durable and potentially curative effects that are already improving human quality of life for patients who have no other available therapeutic options. Until 2021 products report in regenerative medicine field included three main branches of gene therapy, cell therapy and tissue engineered based therapy. But after that time, cell-based immuno-oncology (IO) has emerged in categories independently. Gene therapy and cell-based IO developers raised the vast majority of investment, generating $10.2B and $10.1B in 2021, respectively. However, investment in cell-based IO companies is growing at a faster pace than gene therapy financing. There is no reason except breakthrough treatment for cancer patients and their promising results. According to the latest reports, there are numerous clinical trials in different phases and achieved unprecedented bench to bedside clinical success. So, related market authorized products are increasing subsequently. There are six FDA approved CAR T cell therapy products. Also, there are a superiority in the number of this type of candidate products in the banner year of regulators' Regenerative Medicine Advanced Therapy (RMAT) designation such as FDA and EMA.

Is-17: MicroRNA control of ground-state pluripotency

Moradi Sh

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sh.moradi@royan-rc.ac.ir

Ground-state pluripotency is a stem-cell state achieved through repression of endogenous differentiation processes. The behavior of embryonic stem cells (ESCs) is influenced by ESC-associated microRNAs (miRNAs). Here, we report a comprehensive analysis of the "miRNome" of ESCs maintained under conditions favoring ground-state pluripotency. Using small-RNA sequencing, we also determine the time-resolved profiles of miRNAs over the course of ground-state ESC derivation. We find that ground-state ESCs express a specific set of miRNAs compared to ESCs grown in serum. Interestingly, the majority of ground-state miRNAs are encoded by the imprinted Dlk1-Dio3 locus. Moreover, miRNAs, including the Dlk1-Dio3 miRNAs, are dynamically regulated during ESC derivation. Functional analysis reveal that Dlk1-Dio3 locus-embedded miRNAs (miR-541-5p, miR-410-3p, and miR-381-3p), miR-183-5p, and miR-302b-3p promote, while miR-212-5p and let-7d-3p inhibit ESC generation and/or maintenance. Collectively, these findings offer new mechanistic insights into the role of miRNAs during ESC derivation.

Is-18: An Engineered Multicellular Stem Cell Niche for Studying Disease, Aging and Regeneration

Mashinchian O

Nestlé Research, Nestlé Institute of Health Sciences, Lausanne, Switzerland
Email: Omid.Mashinchian@rd.nestle.com

Fate decisions in the embryo are controlled by a plethora of microenvironmental interactions in a three-dimensional niche. Different bioengineering strategies, such as heterotypic suspension embryoids, organoids, and encapsulation technologies, have been developed and utilized in our group to determine if aspects of this microenvironmental complexity can be engineered to mimic stem cell niches and direct stem cell differentiation. For example, we established a method for the encapsulation of human and mouse skeletal muscle progenitors in diffusible polyethersulfone hollow fiber capsules that can be used to profile systemic aging independent of heterogeneous short-range tissue interactions. Due to the intrinsic complexity of tissues, it remains challenging to pinpoint niche-independent effects of circulating factors on specific cell populations. This method allows us the characterization of effector pathways of systemic aging with unparalleled accuracy. As another example, we introduced a novel means for the generation of large quantities of human myogenic progenitors in a biologically faithful 3D niche environment using an organotypic setting. The scalability of organoid cultures allows for production at the bioreactor level and, next to much-needed cell therapy applications for genetic skeletal muscle diseases, will allow for extensive *in vitro* modeling and screening applications.

Is-19: Antibacterial Host-Guest Curcumin Intercalated LDH/Polyurethanes for Accelerated Dermal Wound Healing

Mohammadi A

Department of Chemistry, University of Isfahan, Isfahan, Iran
Email: a.mohammadi@sci.ui.ac.ir

Is-20: Antibacterial Host-Guest Curcumin Intercalated LDH/Polyurethanes for Accelerated Dermal Wound Healing

Abdolvand H[1], Mohammadi A[1*], Ayati Najafabadi SA[2], Beigi-Boroujeni S[3], Nejaddehbashi F[4], Daemi H[5], Makvandi P[6]

1. Department of Chemistry, University of Isfahan, Isfahan, Iran
2. Department of Biomaterials, Tissue Engineering and Nanotechnology, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. School of Engineering and Sciences, Tecnologico de Monterrey, Av. Eugenio Garza Sada Sur, Monterrey, N.L., Mexico
4. Cellular and Molecular Research Center, Medical Basic Sciences Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

5. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
6. Istituto Italiano di Tecnologia, Centre for Materials Interface, Viale Rinaldo Piaggio 34, Pontedera, Pisa, Italy
Email: a.mohammadi@sci.ui.ac.ir

Curcumin has a limited clinical application due to its extremely low bioavailability. In this study, to increase the bioavailability of curcumin within a castor oil-based polyurethane/LDH wound dressing, an inclusion complex of curcumin and p-sulfonated calix[4]arene (SC4A) was prepared. Then utilized to intercalate the MgAl–layered double hydroxides (LDHs). The introduction of the nanostructure into the PU film (PU/Cur-SC4A-LDH) provided the bacteria-killing performance against both S. aureus and E. coli bacteria. This finding is due to the enhanced bioavailability of curcumin in the PU matrix. Furthermore, all PU nanocomposites exhibited appropriate cytocompatibility based on MTT assay. Mainly, the L929 cells proliferation on the PU/Cur-SC4A-LDH film was significantly further enhanced than other nanocomposites within 7 days. This observation can be related to the better availability of curcumin on the film's surface, which causes an improvement in the proliferation rate of cells. Regarding the histological results, the H&E images showed the faster epidermal layer formation and a more significant number of matured hair follicles for PU/Cur-SC4A-LDH-treated wounds compared to the negative control during 28 days. Thus, this practical healing ability of the PU/Cur-SC4A-LDH nanocomposite makes it a promising candidate as a wound dressing film.
Keywords: Calix[4]arens, Curcumin, Host-Guest Chemistry, Layer Double Hydroxide (LDH), Wound Dressing, Polyurethane Elastomer

Is-21: Alternative 3D Matrix for *Ex Vivo* Expansion of Patient-Derived Bladder Organoids and Tumoroids: Assessment and Characterization

Mollapour Sisakht M[1, 2]

1. **Department of Biochemistry, Erasmus University Medical Center, Rotterdam, The Netherlands**
2. Department of Stem Cell and Regenerative Medicine Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran
Email: mahsamollapur@yahoo.com

Organoid as an aggregation of adult stem cell, progenitor of cells and induced pluripotent stem cell can recapitulate organ function in miniature. This technology become interest during last decade and have been developed for different organs. Organoid are very promising for personalize and regenerative medicines. However, organoids are currently cultured in matrigel which is prepared from the secretion of Engelbreth-Holm-Swarm mouse sarcoma cells. Matrigel is complex, expensive and also poorly defined, with high batch to batch variation. Besides, the xenogeneic origin confide the clinical application. Here, we describe using a combination of alginate. Alginate is a natural polymer produced by brown algae commonly used in biomedical applications because of its biocompatibility, low cost, and low cytotoxicity. To the best of our knowledge, this is the first report of culturing, optimizing and characterization bladder organoid and tumoroids in this scaffold. For this purpose, we cultured the patient-derived tissue samples, and assessed them for the ability of grow, sub culturing capability, long term proliferation potential, size, viability and also expression of specific bladder organoid markers contain CK14, CK20, LGR5, Uroplakin III, FOX1A,GATA3,CK5 and CK44 by RT-PCR. The organoid forming efficiency was evaluated by imaging through confocal microscopy for specific bladder organoid markers (CK20 and Uroplakin III) and proliferation marker such as KI67 as well. The results indicate that alginate is very promising for human bladder organoid culture and has the potential to be used in a variety of clinical applications as well as culturing other types of organoids, especially for the low resources situation.
Keywords: Alginate, Bladder, Organoid, Tumoroids

Is-22: Design and Development of 3D *In Vitro* Models in Cardiovascular Tissue Engineering and Regenerative Medicine

Mostafavi E

Stanford Cardiovascular Institute/Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
Email: ebimsv@stanford.edu

Cardiovascular disease (CVD) is the most leading cause of mortality in the USA and costs $300 billion per year. Due to the lack of appropriate and reliable *in vitro* models to recapitulate the complex structure of the heart organ (from the early stages of development to a mature organ), our understanding is still limited, and we need more well-developed models to be able to accurately investigate the ultrastructural, functional, histochemical, molecular analysis, and gene expression characteristics of the heart. Moreover, the transplantation of bioengineered tissues that can adequately and simultaneously meet the biochemical, electrical, and mechanical demands of the native heart tissue to promote regeneration (for example, following myocardial infarction: MI) is hindered due to the lack of angiogenesis. Integration of nanotechnology, advanced biomaterials, and biology can result in the design and development of *in vitro* models (such as cardiac patches, 3D bioprinted constructs, cardiac organoids, vascular grafts, bioengineered heart tissues, etc.) that can successfully overcome some of the key limitations that can impede the translational applications of them.
Keywords: Biomaterials, Cardiovascular Diseases, Myocardial Infarction, Nanomedicine, Nanomaterials, Regenerative Medicine, Tissue Engineering

Is-23: Characterization of Extracellular Vesicles Mimetic-Nanovesicles and Its Therapeutic Potential

Nasiri Kenari A[1, 2]

1. La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Australia
2. Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Denmark
Email: nasiri.a.aa@m.titech.ac.jp

The therapeutic potential of extracellular vesicle mimetic nanovesicles (M-NVs) has been demonstrated in multiple studies. However, comprehensive characterization and to what extent M-NVs components mimic extracellular vesicles (EVs) remain elusive. M-NVs can be generated through the semi-synthetic method by modifying EVs or fully synthetic by generating nano-size vesicles from cells or individual molecules. In this study, we generated M-NVs from cells through the fully synthetic method, comprehensively characterized and compared

the proteomic and transcriptomic profiles of M-NVs to EVs, and then investigated the therapeutic potential of M-NVs. The proteomic analysis showed that M-NVs contain proteins from subcellular organelles and some EV-enriched proteins, yet distinct from EVs. RNA analysis revealed that M-NVs carry large and heterogeneous RNA, with RNA profiles similar to cells compared to EVs. Next, we encapsulated the miRNA of interest into M-NVs during the extrusion process and showed that the modified M-NVs mediated the functional impact in the recipient cells. In summary, results from this study provided key insight into MNVs protein and RNA composition and demonstrated its therapeutic potential.

Is-24: Hepatocyte-Like Cells: The Good, The Possible, and The Impossible

Nüssler A

Department of Traumatology, Siegfried Weller Institute, BG Clinic, Eberhard Karls University Tübingen, Tübingen, Germany
Email: andreas.nuessler@med.uni-tuebingen.de

Knowledge of stem cells and their use in regenerative medicine has increased tremendously over the past few decades, and many expectations have increased about their use to correct many human pathologies, including many liver diseases. Although many liver-like cell types derived from various stem cell sources have been shown to have hepatocyte/liver function, the majority of these generated cells show only very limited hepatocyte function and their application in clinical trials remains unclear. Stem cells have allowed us to learn a lot about liver development and liver disease or disease etiology, but only limited successes have been published for clinical trials - so I wonder if it isn't time to explore new approaches for the successful use of liver-like cells in research or even to reconsider the approaches in the clinic? The lecture will address both: the knowledge already gained about stem cells in their potential to differentiate into hepatocyte-like cells, but also critically consider the limited clinical success.

Is-25: Electrospun Composite Structures for Tissue Engineering: Challenges and Ppportunities

Pezeshki-Modaress M

Burn Research Center, Iran University of Medical Science, Tehran, Iran
Email: pezeshky.iran@gmail.com

Electrospun nanofibers for tissue engineering applications face two main challenges. The low thickness of electrospun mats and limited cell penetration. The low thickness is the main reason for their weak load-bearing performance at clinical applications and the limited cell penetration is due to their low fiber-fiber distance during deposition process of nanofibers on collector at electrospinning. We have developed different strategies for improving these limitations of electrospun nanofibers in tissue engineering constructs by developing composite structures from different natural and synthetic biopolymers. Fabrication of multi-layered nanofibrous 3D scaffolds comprising chondroitin sulfate by electrospinning method and attaching layers via non-solvent procedure is introduced as an effective method to enhance the applicability of nanofibers for tissue regeneration applications. While nanofibers prepare an ECM-like substrate for cell attachment and proliferation, the gap between layers provides sufficient cellular infiltration and neovascularization. Core/shell composite nanofibers is another suggestion which could offer significant advantages over monolithic fibers especially for enhancing the mechanical performances. Different nature of core/shell structured nanofibers admits the on-demand features in tissue engineering, drug delivery, and dressing applications.

Is-26: HUB Organoids: A Translatable Patient-Derived *In Vitro* Platform for Disease Modelling and Drug Development

Pourfarzad F

Hubrecht Organoid Technology, Disease Modeling and Toxicology Department, Utrecht, The Netherland
Email: f.pourfarzad@huborganoids.nl

Is-27: Intercellular transfer of proteins and RNA mediated by cell-cell contact

Schekman R

Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, United States
Email: schekman@berkeley.edu

A challenge in genome editing in vivo is to devise an efficient means of delivering editing functions, preferably by a vehicle that evades immune detection. We sought a means to deliver Cas9 and a gRNA enclosed within an natural extracellular vesicle as a vehicle for efficient and targeted gene editing. Cas9 was expressed in a donor cell tethered noncovalently to an integral membrane protein, CD63, enriched in exosomes. Exosomes highly enriched in Cas9 and a gRNA were isolated by buoyant density. Isolated exosomes were incubated with reporter cells containing an integrated copy of N-luciferase behind a site which when edited would allow the expression of luciferase. In a control experiment, expression of the Cas9/gRNA construct directly in the reporter cell elicited a 60-70 fold increase in luciferase expression. Exosomes containing a similar level of Cas9 elicited no more than a 50% increase above the background of luciferase. The same was true of conditioned medium containing Cas9-exosomes and even of donor and acceptor cells incubated together separated by a vesicle-permeable membrane in a transwell chamber. In contrast, donor and acceptor cells cocultured to near confluence showed a 60-fold increase in luciferase expression. Transfer of Cas9 appears to be mediated by open-end membrane tubular connections, likely dependent on membrane fusion at the point of junction between a tubule from one cell and the target. A molecular dissection for the requirements for this transfer may permit the development of an efficient means for targeted delivery of Cas9/gRNA

Is-28: Therapeutic Applications of Cell Culture-Derived Extracellular Vesicles: Different Sources, Different Subpopulations

Shekari F

Department of Stem Cells and Developmental Biology Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: f.shekari@royan-rc.ac.ir

Cell culture-derived conditioned medium (CCM) is a valuable source for the isolation of extracellular vesicles (EVs) specifically for therapeutic applications with or without drug loading. We have used different sources of cells including mesenchymal stromal cells (MSCs), fibroblast, and parasite cells to isolate EVs. Following characterization, these EVs have been extensively used to study their therapeutic potential in various diseases. Moreover, the proteome profile and *in vitro* test of different subpopulations of MSC-EVs confirmed that these EVs are molecularly and functionally distinct. The pharmaceutical potency of these EVs has been investigated as a drug delivery vehicle and compared with EVs isolated from milk and red blood cells. Finally, we found that based on the source, the therapeutic potential and pharmaceutical properties of isolated EVs might be different and should be considered rigorously before application.

Is-29: Using Stem Cells to Make Pancreatic Organoids

Tahamtani Y[1, 2]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: y.tahamtani@royan-rc.ac.ir

Large scale generation of functional and vascularized pancreatic organ-like structures from human stem cells will facilitate understanding human pancreas developmental biology. Achieving to such an *in vitro* tool could also be a useful for drug-screening or therapeutic applications for diabetes. However, mechanical and chemical properties of microenvironment, cell types and ratios are important parameters that pose challenges in production of functional pancreatic organoids. Furthermore, organoid formation process and further differentiation *in vivo* can be completely different from *in vitro*. During the earliest stage of pancreas organogenesis, the pancreatic epithelium evaginates into the surrounding non-epithelial niche; Cellular components of the niche secret different growth factors for pancreatic progenitor's (PP) expansion and subsequent differentiation.
Therefore, for the last decade, we have studied combinations of different stem cells with human pluripotent stem cell derived pancreatic progenitors. We investigated the effect of supportive cells as well as different culture conditions in formation of functional pancreatic organoids. During these studies, we developed PP differentiation protocols and co-culture system to study the effects of hESC derived mesenchymal stem cells (MSC), endothelial cells as well as human fetal pancreas mesenchymal cells (hFP-MCs) and bone marrow derived MSC on pancreatic organoids/spheroids formation. Produced structures were also evaluated *in vitro* and *in vivo* for different functional parameters.
We found that cell density/type/ratio are important parameter in self-organization process and organoid formation and we reported that niche-specific stem cells (hFP-MCs) can facilitate further differentiation of PP through a scalable co-culture system. These findings can help developing pancreatic/islet-like organoids for diabetes cell replacement therapies and drug discovery.
Keywords: Embryonic Stem Cells, Organoids, Pancreatic Differentiation, Pancreatic Fetal Mesenchyme, Pancreatic Progenitor

Is-30: Extracellular Vesicles in Chagas Disease (Infectious Disease)

Trocoli Torrecilhas AC

Departamento de Ciências Farmacêuticas, Laboratório de Imunologia Celular e Bioquímica de Fungos e Protozoários, Federal University of Sao Paulo (UNIFESP), Diadema, Brazil
Email: ana.torrecilhas@unifesp.br

Chagas disease, caused by the protozoa parasite Trypanosoma cruzi, is a neglected tropical disease and a major public health problem affecting more than 6 million people worldwide. The diagnosis presents several limitations, the two available treatments cause several side effects and present limited efficacy during the chronic phase of the disease. In addition, there are no preventive vaccines or biomarkers of therapeutic response or disease outcome. Trypomastigote form and T. cruzi infected cells release extracellular vesicles (EVs), which are involved in cell-to-cell communication and can modulate the host immune response. We have shown that vesicles released by infective forms of Trypanosoma cruzi modulate the inflammatory response of macrophages through the activation of Toll 2 receptor (TLR2) involving signaling pathways of mitogen-activated protein kinases (MAPK). This induces the production of nitric oxide (NO) and the cytokines TNF-α, IL-12 and IL-6, which could explain increased inflammation and the progression of disease in experimental models, and eventually the progression of human Chagas' disease. We also found that this process several among different isolates of the parasite that produce different patterns of infection. Furthermore, the vesicles released by the parasite are heterogeneous and different vesicle populations act during the interaction with host cells. We characterize the different populations released by trypomastigotes forms of T. cruzi (Y strain) by Asymmetric flow field-flow fractionation (AF4) and Nanoparticles tracking analysis (NTA) and characterization and to evaluate their interaction with host cells, particularly human monocytes cells (THP-1) and parasite. We also determine the intracellular signaling pathways and the produced cytokines in each case. In Conclusion, our results could help to better understand the mechanism by which the distinct populations of vesicles released by the parasite acts on the communication and modulation parasite-human host cells interaction.

Is-31: Use of Lung Organoids to Model Development and Disease E*x Vivo*

Vazquez-Armendariz AI

Department of Medicine V, Cardio-Pulmonary Institute (CPI), Universities of Giessen and Marburg Lung Center (UGMLC), Giessen, Germany
Email: Ana.I.Vazquez-Armendariz@innere.med.uni-giessen.de

During the last years, organoid models have become a powerful tool to study lung development and disease. Our research focus on the use of three-dimensional lung organoid systems from adult somatic stem cells and induced pluripotent stem cells (iPSCs) for modeling of lung development and disease aiming at the establishment of novel therapeutic strategies. For instance, we established a 3D murine bronchioalveolar lung organoid (BALO) model that allows clonal expansion and self-organization of FACS-sorted bronchioalveolar stem cells (BASCs) upon co-culture with lung-resident mesenchymal cells. BALOs yield a highly branched 3D structure within 21 days of

culture, mimicking the cellular composition of the bronchioalveolar compartment as defined by single-cell RNA sequencing and fluorescence as well as electron microscopic phenotyping. Additionally, BALOs support engraftment and maintenance of different cell types thereby open numerous new avenues to study lung development, infection, and regenerative processes *in vitro*.

Is-32: Novel Aapproach to HCC, How to Attenuate Cancerous Phenotype?

Vosough M

Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: m.vosough@ royan-rc.ac.ir

Background: Growing number's of hepatocellular carcinoma and recurrence frequency have drawn researchers' attention to alternative approaches. The concept of differentiation therapy relies on inducing differentiation in HCC cells in order to inhibit cancerous phenotype. Hepatocyte Nuclear Factor 4 alpha is the key hepatogenesis transcription factor and its upregulation may decrease cancerous features by suppressing epithelial-mesenchymal transition. This study aimed to evaluate the effect of conjugated Linoleic acid (CLA) treatment, natural ligand of HNF4α, on the proliferation, migration, and invasion capacities of HCC cells in vitro.
Materials and Method: Sk-Hep-1 and Hep-3B cells were treated with different doses of CLA or BIM5078 [1-(2′-chloro-5′-nitrobenzenesulfonyl)−2-methylbenzimidazole], an HNF4α antagonist. The expression levels of HNF4a and EMT related genes, were evaluated and associated to hepatocytic functionalities, migration and colony formation capacities.
Results: In both HCC lines, CLA treatment induced HNF4α expression in parallel to significantly decreased EMT marker levels, migration, colony formation capacity, and proliferation rate, whereas BIM5078 treatment resulted in the opposite effects. Moreover, CLA supplementation also upregulated ALB, ZO1, and HNF4α proteins as well as glycogen storage capacity in the treated HCC cells.
Conclusion: CLA treatment can induce hepatocytic differentiation in HCC cells and attenuates cancerous features. This could be as a result of HNF4a induction and EMT inhibition.
Keywords: Conjugated linoleic acid, Differentiation therapy, Epithelial-Mesenchymal Transition, HNF4α ligand, Hepatocellular carcinoma

Is-33: Advanced Microfluidic Systems for Cell Sorting and Single Cell Analysis

Warkiani M

School of Biomedical Engineering, University of Technology Sydney, Sydney, Australia
Email: Majid.Warkiani@uts.edu.au

Micro/nano-fluidics, a technology characterized by the engineered manipulation of fluids at the micro/nano-scale, has shown considerable promise in point-of-care diagnostics and clinical research. Micro/nano-fluidic platforms are creating powerful tools for cell biologists to control the complete cellular microenvironment, leading to new questions and new discoveries. By simply miniaturizing macroscopic systems and taking advantage of the possibility of massive parallel processing, some micro/nano-fluidic chips enable high-throughput biological experiments such as cell sorting, single cell analysis, PCR, ELISA and chromatography. Over the past 15 years, my group has developed several microfluidic systems, which are translated into practice. In this seminar, I will describe our recent efforts in development of new microfluidic systems using 3D printing and microfabrication for high-throughput cell sorting and analysis (e.g., stem cells, CAR-T Cells, CHO cells, etc.) for various applications. I will showcase our new droplet microfluidic tools for single cell analysis too.

Poster Presentations

Ps-1: Contribution of MicroRNA Biogenesis Pathway to Carcinogenesis: A Comprehensive Study Based on Gene Expression Profiles from TCGA Database

Abdi Z[1*], Yeganeh M[2], Kabiri M[1], Moradi Sh[2]

1. Department of Biotechnology, College of Science, University of Tehran, 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
EmailS: sh.moradi@royan-rc.ac.ir, mkabiri@ut.ac.ir

Objective: Cancer cells exhibit numerous deregulations in various genes, including those involved in the microRNA biogenesis pathway. Although cumulative evidence highlights the roles of microRNAs in cancer, the importance of the microRNA biogenesis machinery and consequently the significance of global microRNA activity has not yet been completely surveyed. Here, we aim to explore the expression alterations of microRNA biogenesis components in the deadliest and most prevalent cancers.
Materials and Methods: Raw counts of RNA-sequencing profiles of 13 cancer types were downloaded from The Cancer Genome Atlas (TCGA). The obtained data were normalized using the DESeq2 package (R programming). The normalized counts for 16 genes of microRNA biogenesis machinery were subjected to several analyses including principal component analysis (PCA), Volcano plotting, heatmap analysis, and differential expression analysis. Genes with an adjusted P value<0.05 were considered differentially expressed.
Results: Our results indicated all 16 genes were differentially expressed across different tumor types. The auxiliary components of the microRNA biogenesis pathway (e.g. GEMIN4 and TNRC6A) displayed lower variations in expression compared to the main, canonical components. The key components (i.e. DICER, DROSHA, TRBP, AGO2, and XPO5) were the most differentially expressed (all except DICER were upregulated) and exhibited consistent expression patterns in different cancer types. Interestingly, upregulation of the shuttling components, XPO1 and XPO5, suggested an increased rate of microRNA processing in cancer.
Conclusion: The canonical components of the microRNA biogenesis pathway were the most differentially expressed genes from the microRNA machinery and showed reproducible expression dynamics across various cancer types. All components but DICER were upregulated across various tumor types.
Keywords: Noncoding RNA, Tumorigenesis, In Silico Analysis, Gene Regulation

Ps-2: The Therapeutic Effect of Human Stem Cell Extract on The Healing of Rat Skin Wounds

Abdollahpour A[1*], Safavi Khlkhali SE[1], Amoghli Tabrizi B[2]

1. Department of Pathobiology, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Email: abdollah74.dvm@gmail.com

Objective: A wound is a disorder in the natural structure, purpose, and interior soft layer of skin. The point of healing jagged parts of our skin has been under the studying of scholars and researchers with minimum side effects. There have been new methods of treatment originating from stem cells that aimed to rebuild and heal some tissues and these cells have shown their efficacy in healing animal diseases too. Stem cells with their Prodigious capabilities of propagation and rehabilitation are unique to other kinds of cells. The current survey is aimed at the efficacy of stem cells to treat a mouse wound.
Materials and Methods: The first step was to separate and seed stem cells of the Wharton jelly Umbilical cord. In the next step, 30 Wistar mice were grouped in a pair of 3 groups with the first group (normal), and second group (control) jagged as 1 group included extract 10% division used. This injection was performed on interior tissue in 1 cc around the wound with physiology serum group and with examination group was cell extract. After 21 days some tissue models were gathered. The process of staining by Haematoxylin and Eosin was conducted and histopolytical studies were taken into account.
Results: According to the results the speed of cured wound contraction with cell extract was the most in contrast to other groups. The variation of contraction speed was meaningful (P value<0.05). In association with histopathological parameters in all treated groups with cellular extract, the number of fibroblasts and inflammatory cells and the rate of angiogenesis is more than in the control groups which from a statistical perspective, this difference was meaningful (P value<0.05). The thickness of the germ tissue in the treated group was more than the control group and this difference was also meaningful (P value<0.05).
Conclusion: Totally, the use of Human Wharton's jelly mesenchymal stem cells extract.
Keywords: Cellular Extract, Mesenchymal Stem Cell, Skin Wound, Umbilical Cord, Wharton's Jelly

Ps-3: Sex Differences in The Effect of Long-Term High-Fat Diet on Brain Glucose Metabolism and Expression of Alzheimer's Related Genes in Wistar Rats

Abedi Koshalshahi A[1*], Foroutan T[1], Mohaghegh Shalmani L[2], Dargahi L[3]

1. Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
2. Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Tehran Medical Sciences Islamic Azad University, Tehran, Iran
3. Neurobiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: abedi.masoomeh93@gmail.com

Objective: High-fat diet is reportedly associated with insulin resistance, neuroinflammation, and blood-brain barrier impairment, all postulated as risk factors for Alzheimer's disease. This study investigates whether a long-term high-fat diet (HFD) causes glucose metabolism changes and Alzheimer's-like pathology in a sex-dependent manner in rats when compared with normal chow diet (NCD) intake.
Materials and Methods: After weaning, 20 male and female rats were randomly fed HFD (60% fat) or NCD (10% fat) for

16 weeks. Behavioral tests for spatial memory assessment and 18F-FDG-PET-scan were performed in the final week of experiments. All rats were then euthanized and hippocampal tissues were collected to assess gene expression by qPCR.
Results: Evaluating spatial learning and memory by Morris water maze showed that while the effect of training is significant in all the four groups, memory retrieval measured by the latency to find the hidden platform and the time spent in the target quadrant, is impaired in HFD male rats compared to the corresponding control. In the Y-maze test, HFD male rats showed a significant decrease in spontaneous alternation and an increase in alternate arm returns. In addition, measuring 18F-FDG uptake in the whole brain revealed a significant reduction in glucose metabolism in male but not female HFD rats. Analysis of 8 candidates Alzheimer's related genes including InsR, GLUT1, IDE, BACE1, APP, PSEN1, PSEN2, and BDNF showed that hippocampal expression of GLUT1 and IDE was significantly changed only in HFD male rats.
Conclusion: Our results suggest that sex interferes with the high-fat diet-induced dysregulation of brain glucose metabolism and memory impairment.
Keywords: Alzheimer's Disease, Brain Glucose Metabolism, High-Fat Diet

Ps-4: Leucine and Endurance Exercise Altered The Hub Genes and LncRNAs Networks in Brain-Gut Axis of Mice with Depression Like Behaviors

Abedpoor N[1*], Taghian F[1], Hajibabaie F[2]

1. Department of Sports Physiology, Faculty of Sports Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
2. Department of Physiology, Medicinal Plants Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
Email: f_taghian@yahoo.com

Objective: In developing and developed countries, psychological disorders have imposed heavy economic and social burdens on health care systems. Depression is a frequent mood disorder that might impair the brain-gut axis.
Materials and Methods: Artificial intelligence indicated a mRNAs-lncRNAs network involved in the depression condition. In-silicon and chemoinformatics analysis revealed that four genes, VEGF-α, KDR, PTEN, and BDNF, with the highest degree and most betweenness, could be affected by protein-protein interaction networks under influencing endurance exercise and leucine consumption. We divided 30 mice into five groups: untreated mice, mice with depression-like behaviors, mice with depression-like behaviors treated with consumed leucine, mice with depression-like behaviors treated with exercise training, and mice with depression-like behaviors treated with exercise training along with consumed leucine.
Results: According to artificial intelligence biological analysis, we found some mediators such as lncRNAs profile and Kdr/Vegf-α/Pten/Bdnf interaction network in the hippocampus region and ileum tissue which could be decisive molecules in the brain-gut axis. Moreover, KDR as a principal cutpoint protein in the network was identified as the pharmaceutical approach for major depressive ameliorating based on pharmacophore modeling and molecular docking outcomes. Furthermore, we indicated that the mRNA level of the Pten enhanced and Vegf-α/Kdr/Bdnf mRNAs decreased in mice with depression-like behaviors. Moreover, exercise and leucine ameliorated the brain-gut axis in mice with depression-like behaviors.
Conclusion: Exercise and leucine regulated the lncRNAs network in the hippocampus and ileum of mice with depression-like behaviors. We suggest that the lncRNAs profiles could consider as diagnosis and prognosis biomarkers, and exercise + leucine might be a practical approach to improve depression.
Keywords: Brain-Gut Axis, Depression, Endurance Exercise, lncRNAs, Leucine

Ps-5: Human Umbilical Cord Blood-Derived Serum for Culturing Human Mesenchymal Stromal Cells

Afzal E[1*], Pakzad M[2], Nouri M[1], Moghadasali R[2], Zarrabi M[1, 3]

1. Department of Research and Development, Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: m.zarrabi@royan-rc.ac.ir

Objective: In the use of bovine fetal serum (FBS) there is concern about the possibility of disease transmission from animal to human. Therefore, it seems necessary to create culture conditions free of animal serum, especially in cell therapy. This study aimed to evaluate the feasibility of replacing human umbilical cord serum (hUCS) with FBS for *in vitro* expansion of umbilical cord mesenchymal stromal/stem cells (UC-MSCs).
Materials and Methods: Here, UC-MSCs were cultured for five days in media supplemented either by hUCS or commercial FBS (Gibco & HyClone) to compare their viability, proliferation, morphology, Immunophenotype, and differentiation potential.
Results: Notably, the rate of cell proliferation in the group containing 2% hUCS was the same. Our data shows that the use of 5% and/or 10% hUCS, resulted in a 10-fold increase in the number of MSCs; While in the presence of commercial FBS, this figure reached a maximum of five times. as the groups containing 10% commercial FBS. Furthermore, there was no significant difference between groups in terms of viability, surface markers, and multilineage differentiation potential.
Conclusion: These results demonstrated that hUCS can efficiently replace FBS for the routine culture of MSCs and can be used ideally in the manufacturing process of UC-MSCs in the cell therapy industry.
Keywords: Fetal Bovine Serum, Human Umbilical Cord Blood Serum, Mesenchymal Stem/Stromal Cells, Serum Supplement

Ps-6: The Effect of Amniotic Membrane Scaffold on Amniotic Fluid Mesenchymal Stem Cell Growth and Their Differentiation into Neuron Cells

Aksoun M[1*], Azar Afshar Z[2], Nikonahad N[1]

1. Department of Biology, Faculty of Sciences, Science and Art University, Yazd, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: nikoonahad@sau.ac.ir

Objective: Amniotic fluid mesenchymal stem cells (AFMSCs) are isolated from AF during PGD. AFMSCs differentiate to

other cell lines including neuronal cells under standard differentiation conditions. Scaffold allows cells to attach, causes cell migration, cell production, and transfer biochemical factors. AM is the part of the placenta that can be used as a natural scaffold in research.
Materials and Methods: AM was decellularized, cut into small pieces, and placed on a petri dish surface. AF cells were successively passaged, and cultured on Petri dishes with/without scaffold. AFMSCs grew well and the cells were confirmed to be mesenchymal by flow cytometry. They were cultured in a differentiation medium with growth and inducing factors to stimulate nerve differentiation, which was confirmed by real-time polymerase chain reaction (PCR). Lastly, the effect of the AF scaffold on neural differentiation of AFMSCs was assessed using scanning electron microscopy (SEM) imaging.
Results: It was observed that the cells were well adhered to the cell scaffold and their growth was better. These results were confirmed by SEM imaging. Generally, after cell culture on the scaffold and without the scaffold, which was performed in consecutive passages, cell differentiation to neural cells using the scaffold showed a greater increase in differentiation than cell culture without the scaffold. ANOVA analysis also confirmed this observation (P value <0.05).
Conclusion: The findings of this study can be useful in improving the neural differentiation of AFMSCs, which is an accessible and valuable resource. It is also promising to use these cells in reconstructive medicine and in the treatment of nerve tissue and degenerative diseases of nerve cells.
Keywords: Amniotic Fluid, Mesenchymal Stem Cell, Neural Differentiation, Scaffold

Ps-7: COL4A6 and SPARCL1 Have Crucial Role in Neural Cells Therapies Microenvironments with Zinc Finger Transcription Factor Treatment

Arabi M[1*], Bereimipour A[2]

1. Department of Genetics, Medical Genomics Research Center, Tehran Medical Sciences Islamic Azad University, Tehran, Iran
2. Departmet of Molecullar Genetics, Faculty of Science and Advanced Technologies in Biology, University of Science and Culture, Tehran Iran
Email: ahmadlta@yahoo.com

Objective: Cell therapy for nerve cells can be an important factor in treating diseases associated with nerve damage. In the meantime, it is very important to study different conditions and the function of cell therapy in the microenvironment of the cells. In this study, we focused on the role of zinc finger transcription factor in neural tissue cell therapy and its molecular mechanisms.
Materials and Methods: A bioinformatics approach was used to look at the molecular mechanisms and discover important elements between intact neural cells and zinc finger transcription factor. In this study, we looked at genes, protein products, and molecular mechanisms in neural cells and zinc finger treatments utilizing integrated and continuous bioinformatics analytics via multiple tools and databases.
Results: Wnt, axon guidance, focal adhesion, HTLV infection, and immune system signaling pathways were all involved in the findings acquired. When all the information was analyzed, SPARCL1, ANO1, LTBP1, CA12, and COL4A6 proteins were identified.
Conclusion: Finally, in this investigation, neural cell therapy and zinc finger transcription factor treatments with candidate genes had a more robust and better association for microenvironment changes phenomenon.
Keywords: Bioinformatics, Cell Therapy, Microenvironment, Neural Cell

Ps-8: Proliferation of Spermatogonial Stem Cells Cultured in Direct and Indirect Contact of Human Amniotic Mesenchymal Stem Cells

Asgari F[1*], Jahanbakhsh M[2], Koruji M[2, 3], Amoozgar H[1], Asgari HR[2, 3]

1. Clinical Research and Development Center of Nekoie Hedaity Forghani Hospital, Qom University of Medical Sciences, Qom, Iran
2. Stem Cell and Regenerative Medicine Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: asgari.hr@iums.ac.ir

Objective: Spermatogenesis is influenced by chemotherapy and radiography in cancerous patients especially in children who have recovered. This study aimed to compare the effect of direct and indirect contact of human amniotic mesenchymal stem cells (hAMSCs) on the proliferation of mouse spermatogonial stem cells (SSCs).
Materials and Methods: HAMSCs were extracted from placental tissue and co-cultured with SSCs of 3 to 6-day-old mice. SSCs were examined in three experimental groups: control, condition media and insert groups in indirect contact with hAMSCs for two weeks. The quantitative real-time polymerase chain reaction (qRT-PCR) was performed to assess proliferation genes expression. Plzf marker was assessed by Immunohistochemistry (IHC) and C-kit, and PLZF marker was evaluated by flow cytometry.
Results: Flow cytometric analysis confirmed that the Plzf-positive cells reached 2 fold at the end of the 14th day of culture in the media group (76.47, P value $\leq$0.05). Plzf gene expression showed a significant increase in the media group (188.1 $\pm$ 65, P value $\leq$0.05). The C-kit gene showed a significant decrease between both media and insert groups compared to the control group. Imunocytochemistry (ICC) analysis showed a significant difference in Plzf and C-kit markers in all groups compared to the control.
Conclusion: We were able to introduce hAMSC as a new feeder cell source for increasing the proliferation of SSCs, which can aid males with infertility.
Keywords: Amniotic Mesenchymal Stem Cells, Placenta, Proliferation, Spermatogenesis

Ps-9: Fabrication of 3D Bioprinted Scaffold with Biohybrid Aalginate Dialdehyde /Bone Extracellular Matrix for Bone Tissue Engineering

Ashrafnia A[*], Mashayekhan Sh

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

Objective: The right combination of stem cells and reasonable biocompatible materials helps to improve bone regeneration. Bone extracellular matrix (ECM)-derived hydrogels have been

extensively used as a bioactive matrix to expedite the functional regeneration of bone tissue, but low mechanical strength and rapid degradation limit the broad utilization of ECM for clinical applications. Here, we present biohybrid hydrogel based on demineralized and decellularized human bone matrix (DBM) and alginate dialdehyde (AD) for bone tissue engineering.
Materials and Methods: The alginate was partially oxidized with an oxidation degree of 5% and the resulting AD was cross-linked by calcium carbonate. Also, AD aldehyde groups reacted with DBM amine groups through Schiff-base reaction. To determine whether the bio-ink was printable, AD hydrogel with different concentrations of DBM were printed by a three-dimensional (3D) bioprinter.
Results: Bio-ink viscosity increased with increasing DBM concentration and the hydrogels composed of 8% w/v AD and 2% w/v DBM showed acceptable printability. Moreover, the developed bio-ink containing DBM showed a suitable gelation time and tensile Young's modulus. Cytocompatibility analysis of the biohybrid hydrogel was conducted by MTT assay using placenta-derived mesenchymal stem cells (P-MSCs). The results showed that cell growth increased significantly with increasing DBM. Hydrogel with 4% w/v concentration of DBM demonstrated higher cell viability compared with the other samples. The hydrogel containing 2% DBM also showed acceptable cell adhesion and growth.
Conclusion: Overall, according to printability and biocompatibility results, hydrogel with 8% w/v AD and 2% w/v DBM may be considered as the optimal candidate as bio-ink to be applied for further studies in 3D bioprinting for bone tissue engineering. The bio-ink with the above-mentioned composition may provide an appropriate niche for the migration and differentiation of MSCs.
Keywords: Alginate Dialdehyde, Bioink, Decellularized Human Bone Matrix, Placenta-Derived Mesenchymal Stem Cells

Ps-10: Proliferation and Invasion Inhibition of Colorectal Cancer Cells by Knocking Out The MSI1 Gene Using CRISPR/Cas9 Technique

Azimi AH[1*], Dormiani K[2]

1. Department of Biology, Faculty of Science and Technology, ACECR, Institute of Higher Education, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: k_dormiani@royaninstitute.org

Objective: Colorectal cancer is one the most common cancers worldwide. The RNA-binding protein Musashi1 (MSI1) plays an important role in the regulating of the cell differentiation in colon progenitor cells. MSI1 overexpression has been reported in several malignances including colorectal cancer. Overexpression of the MSI1 gene increases cell division and invasion, but decreased expression of MSI1 leads to reduced cell proliferation and invasion in a variety of malignancies. The aim of this study was to knock out the MSI1 gene in colorectal cancer cells by the CRISPR/Cas9 technique and seek its effect on proliferation and invasion rate of the cancer cells.
Materials and Methods: First, appropriate sgRNAs targeting MSI1 gene was designed bioinformatically. HCT116 cells with the highest expression of MSI1 were used for knocking out. Two vectors, one containing the specific sgRNAs sequences, and the other with Cas9 coding sequence were constructed and co-transfected into the HCT116 cells by lipofection. The function of Cas9 in specific deletion of the MSI1 genomic region was determined using polymerase chain reaction (PCR) and sequencing. Finally, the effect of decreasing MSI1 expression on proliferation and invasion of the colorectal cancer cells were investigated.
Results: The accuracy of constructed vectors was confirmed using PCR and sequence analysis. Flow cytometry and fluorescent microscope also showed the successful co-transfection of HCT116 cells due to expression of EGFP and mCherry reporters. The efficient deletion of target sequences was also verified using genomic PCR. Finally, the results showed that colorectal cancer cell proliferation and invasion were significantly reduced compared to untreated cells as the control.
Conclusion: Based on our findings, targeting and disruption of MSI1 as an oncogene could be a promising approach to improve the cytotoxic effect against the colorectal cancer cells.
Keywords: Colorectal Cancer, Crispr/Cas9, Knockout, MSI1 Gene,

Ps-11: Antitumor Effects of Human Induced Pluripotent Stem Cell-Based Cancer Vaccine on Animal Model of Breast Cancer

Badri MS[1, 2*], Zarghami M[1, 2], Saber M[2], Shekari F[2], Ebrahimi M[2], Hassani N[2]

1. Department of Developmental Biology, Faculty of Sciensec 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: sn.hassani@royan-rc.ac.ir

Objective: Studies on cancer as the second leading cause of death, have been indicated the similarity between pluripotent stem cells (PSCs) and cancer cells in cellular and molecular characteristics. This similarity has led to the hypothesis that PSCs could be used as tumor antigens and opened new approaches for the possibility of using PSCs for cancer treatment.
Materials and Methods: The human induced pluripotent stem cell line (CAG-hiPSC) was provided from Royan Stem Cell Bank and cultured. The cells were prepared in lysate form before vaccination. Cell lysate was prepared after five cycles of freeze-thawing. BALB/C mice were immunized in different groups with 1) hiPSC lysate, 2) hiPSC lysate+Adj, and 3) Freund adjuvant (control). After three once-weekly vaccinations, 4T1 breast cancer cell line was injected into the mice. Immunological analyzes were performed to investigate the immune response in different treatment groups.
Results: Immunohistochemistry (IHC) staining revealed that the expression of CD8 and CD4 markers were significantly higher in the tumor of hiPSC+Adj-treated mice in comparison to the Adj-treated mice. Moreover, mice treated with hiPSC+Adj exhibited significantly increased secretion of IFN-γ and IL-4 in comparison to the Adj-treated mice. There were no significant differences in the frequency of IL-10 between hiPSC+Adj and Adj groups.
Conclusion: Our findings suggested that among all the treatments tested, the combination of hiPSC with Adj could provide more effective immunity in the breast cancer mouse model by inducing anti-tumor responses.
Keywords: Antitumor Immunity, Cancer Vaccine, Human Pluripotent Stem Cells

Ps-12: Evaluation of The Anti-Cancer Effect of XBP1s-Decoy Oligodeoxynucleotide in HCC

Bahadori S[1, 2*], Farzaneh Z[1], Solhi R[2], Vosough M[2, 3]

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 Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Experimental Cancer Medicine, Institution for Laboratory Medicine, Karolinska Institute, Huddinge, Sweden
Email: m.vosough@royan-rc.ac.ir

Objective: Hepatocellular carcinoma (HCC) is one of the most frequent tumors in humans. X-box binding protein 1 (XBP1) is a transcription factor (TF) involved in the unfolded protein response, a cell's adaptive response and defense mechanism to survive in severe environments. A growing number of studies demonstrate that XBP1s is commonly constitutively activated in a variety of cancers, including HCC, making it a promising molecular target for cancer therapy. Transcription factor decoys oligodeoxynucleotides (TFD) are a new class of oligonucleotide-based molecular therapeutics that are short, double-stranded DNA. TFD binds to the DNA binding site of a specific gene in a target TF and suppresses TF activity as well as gene expression. In the current study, we aimed to reduce the cancerous phenotype in HCC cell lines by treatment of cells with TFD.
Materials and Methods: We transfected HCC cells with XBP1s-decoy ODN, which selectively blocks over-activated XBP1s, and assessed cellular migratory ability by wound healing assay. Due to the identified viability potent of HCC cells, cell counting assays were performed. For more evaluation, the levels of XBP1s-regulated genes such as BAX, MYC, CYCLIN-D1, MMP-9, and CDH1, which are involved in cell apoptosis, cell cycle, invasion, and migration, were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR).
Results: Transfection of HCC cells resulted in significant down-regulation of MYC, CYCLIN-D1, MMP-9, and up-regulation of CDH1 and BAX at transcription levels. Also, the evaluation of cell counting and wound healing assay suggested that transfection of XBP1s-decoy could reduce cell viability and migration capacity of HCC cells as well.
Conclusion: This idea shows that transfection of XBP1s-decoy ODN can decrease migration and cell viability. This inhibitory effect is accompanied by a reduction and acceleration of our interest genes.
Keywords: Hepatocellular Carcinoma, X-Box Binding Protein 1 (XBP1), Transcription Factor Decoys Oligodeoxynucleotides, XBP1s-Decoy ODN

Ps-13: Targeted Genome Editing by Recombinase-Mediated Cassette Exchange (RMCE) Increases the Productivity of The Chinese Hamster Ovary Cell Line

Bastanifard M[1, 2*], Amiri-Yekta A[2]

1. Department of Molecular Genetics, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: amir.amiriyekta@royaninstitute.org

Objective: The recent development of genome editing tools offered targeted gene integration methods to get control over gene integration positions and outcomes in biopharmaceutical productions and reduce clonal variation. Recombinase-mediated cassette exchange (RMCE) is a powerful strategy for targeted gene integration. Our research aimed to develop and evaluate an engineered CHO cell line platform applying the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) targeted gene integration and Cre/LoxP recombinase system to improve the recombinant protein yield and create predictable expression.
Materials and Methods: In the current research, we generated an engineered CHO-DG44 based cell line using CRISPR/Cas9 technology, including a replaceable cassette (LoxP/mCherry/Lox2272) inserted in an expressional hot spot locus, C12orf35. The modified single cells were verified by borderline polymerase chain reaction (PCR), RT-PCR, and microscopy analysis. Following, the donor vector consisting of Erythropoietin (EPO) ORF, IRES, hygromycin resistance gene flanked by LoxP, and Lox2272 constructed and co-transfected along with the plasmid carrying the Cre-recombinase (pMC-Cre) to the modified CHO-DG44 cells. The transfected cells were selected by a selective media and confirmed by fluorescence microscopy analysis, genomic PCR, RT-PCR, and western blotting.
Results: After transfection, positive cells showing no red signals were picked up by fluorescence microscopy and the integration and mRNA expression were confirmed by PCR and RT-PCR, respectively. Protein expression was confirmed by western blotting. We demonstrated that EPO was expressed in the pellet/SUP of the positive clone/s. Because our donor vector was promoter-less, the CMV promoter was introduced in the target location first, and a positive result for RT-PCR and western blotting showed the correct cassette exchange.
Conclusion: In this research, the targeted integration approach by RMCE assessed a site-specific integration platform on the modified CHO-DG44 cell line that allows controllable and reproducible integration of different recombinant genes. It's a promising strategy for high titer recombinant production based on the next-generation CHO cell factories.
Keywords: Biopharmaceuticals, CHO-DG44, Recombinase-Mediated Cassette Exchange (RMCE), Targeted Gene Integration

Ps-14: Sciatic Nerve Regeneration by Differentiation of Human Uterine Endometrial Derived Mesenchymal Stem Cells into Nerve-like Cells Using Polyacrylonitrile/Chitosan Conduit

Bostani AA[1*], Razeghi J[1], Naddaf H[2], Hoveizi E[3]

1. Department of Plant Sciences, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
Email: Aliasgharbostani98@gmail.com

Objective: One of the prevalent human disabilities is peripheral nerve damage. Sciatic nerve injury is a tremendously useful model for studying nerve regeneration. Today, one of the most modern methods for treating this complication is tissue engineering. In tissue engineering, in addition to stem cells, Conduits are of great importance. Conduits are a substrate for cell attachment, migration, and differentiation in the developing

tissue. One of the considered methods in preparing Conduits is electrospinning. There are many kinds of biomaterials for tissue engineering, including polyacrylonitrile (PAN). Also, Chitosan is a natural polymer that can modify the properties of the Conduit. The present study aimed to use nerve-like cells (NLCs) derived from human uterine mesenchymal stem cells on polyacrylonitrile/chitosan conduit for sciatic nerve regeneration.
Materials and Methods: In this study, the PAN Conduit was prepared by an electrospinning method. The properties of the Conduit were examined by scanning electron microscopy (SEM). Mesenchymal stem cells were extracted from human endometrial tissue (EnMSCs), and their identity was confirmed by flow cytometry. Then EnMSCs were seeded in chitosan hydrogel, cultured in conduit, and differentiated into nerve-like cells using FGF, EGF, and factor B27 for 14 days. Twenty-one male adult Wistar rats (200-250 g) were used in this study. Sciatic nerve injury was performed on all three groups. Groups included: 1- control group without any treatment, 2- conduit, 3- conduit/NLCs. After eight weeks, a sciatic functional index (SFI) and a histological examination were used to determine nerve regeneration.
Results: The results showed that EnMSCs differentiated into nerve cells under our differential condition and the presence of chitosan with significant efficiencies was observed. The expression of markers such as Nestin, Map2, Tuj-1, and NF confirmed neuronal induction in these cells. Also, electrospinning of PAN polymer was a suitable method for preparing a conduit, and EnMSCs were attached to this conduit with a high number and survival. These cells can grow and differentiate on this biocompatible polymer under suitable conditions. In the histological study, the number of axons was higher in the treated groups compared to the control group. Also, a significant difference in the SFI was observed between the conduit/NLCs group and the control group.
Conclusion: The present study showed that using tissue engineering to compensate and treat neurological complications such as peripheral nerve injuries will be effective.
Keywords: Conduit, Mesenchymal Stem Cells, Neural Tissue Engineering,

Sciatic Nerve Injury,

Ps-15: KIR Positive NK Cells Effectively Target Leukemia Cell Lines

Dizaji Asl Kh[1*], Mazloumi Z[2], Rafat A[3], Nozad Charoudeh H[1]

1. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: nozadh@tbzmed.ac.ir

Objective: Acute lymphoid (ALL) and myeloid leukemia (AML) are known to be invasive and highly lethal hematological malignancies. Insufficiency of the present treatments and their diverse side effects lead researchers to find new and more effective therapeutic methods. Interestingly, ongoing efforts to find the best approach to optimize natural killer (NK) cell anti-leukemia potential shed light for the successful treatment of cancer. Mature KIR+NK cells' ability to remove HLA Class I deficient cells has been exploited in cancer immunotherapy.
Materials and Methods: To precisely address the influence of complex cytokines on NK cells development, cord blood derived MNCs and CD34+ cells were seeded with different cytokine combination. NK cell differentiation and their cytotoxic function were examined with KIRs/CD56 and CD107/IFN expression, respectively.
Results: Our finding underlined the importance of KIR expression in cytotoxic function of NK cells. Taken together, this study presented an effective *in vitro* method for the expansion and differentiation of KIR+NK cells using cytokines without any feeder cells.
Conclusion: The presented culture condition could be helpful for the generation of mature and pure NK cells even from limited numbers of CD34+ cord blood cells and might be used as a novel method to improve the current state of cancer therapy.
Keywords: Acute Lymphoblastic Leukemia, Cord Blood Stem Cells, Killer Cell Immunoglobulin-Like Receptors, Natural Killer Cells

Ps-16: Design and Fabrication of Gene Constructs to Generate A Double Transgenic Zebrafish Model for Studying Pancreatic Beta Cell Regeneration

Eghbal D[1, 2*], Rezaei M[2, 3], Tahamtani Y[2, 3]

1. 1. Department of Molecular Genetics, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: y.tahamtani@royan-rc.ac.ir

Objective: There are scientific evidences that show pancreatic alpha cells (pAC) are able to contribute to beta cell (pBC) compensation through diabetic pathophysiologic condition. Zebrafish is a well-suited transgenic animal model for regenerative studies due to easy accessibility to the embryos as well as cell signaling and developmental similarities with mammals. Generating transgenic zebrafish models was done by tol2 transposase system in zebrafish labs.
Materials and Methods: Here, we designed and constructed ins:RFP-NTR and Gcga:eGFP plasmids to generate a double transgenic zebrafish model which demonstrates endocrine cell plasticity through pBC regeneration. Accordingly, ins-RFP-NTR plasmid digested by Age1 and BsrG1 restriction enzymes (RE) and RFP was inserted downstream of insulin (ins) promoter. Second cloning was performed to insert Glucagon (Gcga) promoter into tol2-eGFP plasmid.
Results: Designed primer were used for amplification of 2kb of Gcga promoter to be inserted upstream of eGFP by SalI RE. Recombinant plasmids were confirmed by colony polymerase chain reaction (PCR), digestion, and sequencing; in colony PCR and digestion proper fragment sizes were obtained and no errors were detected through sequencing data. These plasmids, then, will be injected into one-cell embryos to generate Tg(ins:RFP-NTR, Gcga:eGFP) zebrafish. Specific Beta-Cell ablation could be occurred by a fusion protein in ins producing cells called NTR.
Conclusion: Overlap of green and red color was observed which indicates the state transition of Gcga+ to ins+- cells us-

ing confocal fluorescent microscopy. This line could be used for further drug screening experiments and the discovery of new beta regeneration mechanisms.
Keywords: Diabetes, Metronidazole, Primer, Tol2

Ps-17: Short-Time Activation of Retinoic Acid Pathway Leads to Generate Naive-Like Pluripotent Stem Cells

Enteshari Z[1, 2*], Taei A[2], Hassani SN[2]

1. Department of Molecular Genetics, Faculty of 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: sn.hassani@royan-rc.ac.ir

Objective: Compared to primed pluripotent stem cells (PSCs) that represent the prototype of post-implantation epiblast cells, naïve PSCs share traits with the pre-implantation epiblasts. These stages of embryonic development can be imitated *in vitro* by modulating the main signaling pathways involved in pluripotency. We have recently shown that transient activation of retinoic acid receptor gamma (RARɣ) and liver receptor homolog-1 (LRH-1) induces naïve pluripotency in human PSCs. Here, we further investigated the impact of retinoic acid (RA) to the induction of naïve-like PSCs in 2iL medium containing LIF, and chemical inhibitors of GSK3β and MEK 1/2.
Materials and Methods: In this study, we used RA and RARɣ agonist, CD437, for 4 to 5 days to induce primed PSCs into the naïve state. The successful conversion was examined morphologically and by immunostaining for some naïve-specific markers.
Results: The result revealed that the short-term treatment with CD437 or RA causes the formation of naïve-like PSCs. The resulting cells had dome-shaped morphology and were insensitive to single-cell dissociation and ROCKi independent. These cells also showed NANOG expression and nuclear localization of TFE3.
Conclusion: Our results displayed that temporary activation of the RA signaling pathway in combination with 2iL resulted in the induction of naïve-like pluripotency in human PSCs. These results highlight the function of RA signaling in pluripotency.
Keywords: Naïve Pluripotency, Pluripotent Stem Cell, Retinoic Acid, Retinoic Acid Receptor Gamma (RARɣ)

Ps-18: Fabrication and Characterization of A Three-Dimensional Scaffold Using Bovine Extracellular Matrix for Muscle Tissue Engineering

Erfanian S[1*], Kazemi Ashtiani M[1], Rajabi Zellati S[1], Baharvand H[2]

1. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, 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: s.rajabi@ royan-rc.ac.ir

Objective: Native tissues have numerous advantages for application in tissue engineering. For instance, muscle tissue contains significant amounts of extracellular matrix (ECM) components and rich source of endogenous growth factors. In this study, we have developed a muscle-derived scaffold that mimics the natural muscular extracellular environment and characterized its properties for muscular tissue engineering.
Materials and Methods: Firstly, decellularization was performed using Triton X-100, sodium dodecyl sulfate (SDS), and Trypsin /EDTA. The efficiency of the decellularization process was evaluated using DNA content assay, DAPI, Alcian blue, Masson's trichrome, and hematoxylin and eosin (H&E) staining. The hydrogel solubilization procedure was done in acetic acid with pepsin. The 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) solution was used as a cross-linker agent to improve the mechanical properties of three-dimensional (3D) lyophilized scaffold. The physical properties of sponge-like 3D scaffolds were investigated by scanning electron microscopy (SEM), 2, 4, 6-Trinitrobenzenesulfonic acid (TNBS) assay, H&E staining, swelling test, mechanical test, and *in vitro* degradation assay.
Results: Histological analysis of decellularized bovine muscle showed significant removal of cellular components and the preservation of collagen, elastic fibers, glycosaminoglycans, and proteoglycans. Moreover, the DNA content assay confirmed the successful decellularization showing DNA decreasing from 1198.7 ± 289.98 ng/mg to 15.11 ± 17.45. The results of the TNBS test showed that cross-linking efficiency of muscle-derived ECM scaffolds was about 20.86. The average pore size of the 3D crosslinked scaffold was 123.71 ± 55.02 µm measured from SEM images which is suitable for cell penetration, nutrients, and gas change. The obtained compressive (0.093 MPa) and tensile (8.71 MPa) moduli were in the range of values reported for scaffolds intended for skeletal muscle regeneration.
Conclusion: Decellularization of native bovine muscle was performed in this study. The decellularized scaffold has been fabricated by freeze-drying followed by chemical crosslinking. The structure displays suitable pore size, *in vitro* degradation, and mechanical properties which may open new opportunities for the application of muscle tissue engineering.
Keywords: Extracellular Matrix, Muscle Tissue, Scaffold, Tissue Engineering

Ps-19: Investigation of The Gene Expression Profiles of Normal and Injured Podocytes

Esmaeili MR[1], [2*], Nejati M[2, 3], Taleahmad S[2], Moghadasali R[2]

1. Department of Developmental Biology, Faculty of Basics Sciences and Advanced Technologies in Biology, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Cell and Molecular Biology, School of Biology, College of Science, University of Tehran, Tehran, Iran
Emails: s.taleahmad@royan-rc.ac.ir, rezamoghadasali@royaninstitute.org

Objective: Chronic kidney disease affects more than 10% of the world population. 90% of all cases are attributed to glomerular disease. The most sever forms of kidney diseases are often associated with irreversible damage to glomerular podocytes-highly specialized epithelial cells that encase glomerular capillaries and regulate removing toxin and wasted components from the blood. As a consequence, finding signaling pathways related to the podocyte damage by bioinformatics approaches is essential.

Materials and Methods: In this study we focused on gene expression profiling in damaged podocyte cells and investigated signaling pathways associated with this matter. At first, we selected suitable studies from GEO database and analyzed with GEO2R tool. Then uploaded the up/down regulated genes to VENNY and selected processed in common genes between up/down regulated groups to examine the signaling pathways in the DAVID database and the KEGG library. Finally, we used StRING database to select protein interactions.
Results: Results showed that 664 genes up regulated and 394 genes were down regulated. Among those up regulated genes most of them were expressed in NF-κB, TNF, MAPK, and NOD-like signaling pathways, in other hand metabolic and fatty acid degradation signaling pathways were observed in down regulated genes. *Tnf, Egfr, Icam1, Birc3, Ccl5, Nfkb2* genes were up regulated and the results also obtained that *Acadm, Acads, Eci2, Aldh1a3, Cyp26a1* were down regulated.
Conclusion: Finally, by these results we observed the genes that identified in podocyte cells damages that highly related to immunity, inflammation, and metabolic pathways.
Keywords: Chronic Kidney Disease, Gene Expression Profile Kidney, Podocytopathy

Ps-20: Chemically Induced Hypoxia, Enhances Osteogenesis Effect of Human Adipose-Derived Stem Cells in Osteoporosis in Rat Model

Fallahnezhad S*

Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: fallahnezhads@mums.ac.ir

Objective: Osteoporosis (OP) is a chronic progressive metabolic bone disease caused by the reduced bone formation and increased bone resorption. Recently, applying mesenchymal stem cells (MSCs) has gained a huge amount of interest among scientists. Hypoxic preconditioning (HP) of MSCs is an efficient strategy of reducing apoptosis of the stem cells and enhancing their regenerative ability and therapeutic potentials for the treatment of OP. The present study aimed to examine the effect of injection of human adipose-derived MSCs pre-conditioning with Cobalt Cholride on the osteogenesis in Wistar ovariectomized rats.
Materials and Methods: In this study, osteoporosis was induced by bilateral ovariectomy. In the pilot study, human adipose-derived MSCs were pre-conditioned with 100, 200 and 400 μM cobalt chloride for 24 and 48 h. According to MTT and Acridine Orange Ethidium Bromide staining results, 100 μM cobalt chloride for 48 h was used to continue working. Four months after ovariectomy, rats were then divided into three groups receiving pre-conditioned hypoxic cells, normoxic cells and saline buffer phosphate. At 12 and 16 weeks, sampling was performed to evaluate the expression of Runx2, OPG and OC proteins in tibia by western blotting.
Results: The results showed that in the group receiving hypoxic cells in both weeks 12 and 16, the expression of Runx2, OPG and OC proteins was significantly increased compared to the sham and normoxic groups.
Conclusion: Concluded that artificially controlled direction of MSC migration could remodel the microstructure of bone tissue, which led to the appropriate osteogenesis.
Keywords: CoCl2 , Human Adipose-Derived Stem Cells, Osteogenesis, Osteoporosis

Ps-21: In A Rat Model of Cerebellar Ataxia, Human Olfactory Epithelium-Derived Stem Cells Improve Histopathological Impairments

Fathi M[1*], Vakili K[1], Aliaghaei A[2]

1. Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Brain Mapping Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: mobina.fathi78@gmail.com

Objective: Cell replacement therapy (CRT) is one of the most effective treatments for symptoms of neurodegenerative diseases including cerebellar ataxia (CA). Due to their particular qualities, such as immunomodulatory activities and ease of access compared to other types of mesenchymal stem cells (MSCs), human olfactory epithelium mesenchymal stem cells (OE-MSCs) have been identified as a prospective candidate for CRT. As a result, the main purpose of our research was to see how OE-MSC transplantation affected behavioral, structural, and histological defects in a rat model of CA.
Materials and Methods: OE-MSCs were extracted from individuals' nasal cavities after they gave their informed consent. The positive expression of CD73, CD90, and CD105 as MSC markers, as well as nestin and vimentin as primitive neuroectodermal stem cell markers, were used to identify OE-MSCs. The rats were then randomly assigned to one of the three groups: control, 3-acetylpyridine (3-AP) treated, or 3-AP+ cell. The rats were given an intraperitoneal dose of 3-AP (75 mg/kg) in both experimental groups, followed by the implantation of OE-MSCs into the 3- AP+ cell group's cerebellum. The effect of engrafted OE-MSCs on motor coordination and performance, as well as biochemical, immunohistochemical, and stereological changes in the cerebellum of CA-prone rats, was studied.
Results: The delivery of 3-AP reduced the concentration of GSH in the cerebellum, according to our findings. The morphological properties of the cerebellar Golgi cells were similarly affected by 3-AP injection. OEMSC transplantation, on the other hand, enhanced motor coordination in CA. Furthermore, when 3-AP was administered, OEMSCs inhibited the caspase-3 expression and microglia proliferation in the cerebellum. Finally, Purkinje cells were protected from 3-AP toxicity by OEMSC transplantation.
Conclusion: In conclusion, the current study found that OE-MSCs have significant benefits in the CA animal model.
Keywords: Caspase-3, Cerebellar Ataxia, Olfactory Stem Cell

Ps-22: Fabrication and Evaluation of Piezoelectric Poly (L-Lactide)-Based Electrospun Scaffold for Bone Tissue Regeneration

Fotoukian HR[1, 2*], Haghbin Nazarpak M[1], Solouk A[2], Nouri AR[2]

1. New Technologies Research Center (NTRC), Amirkabir University of Technology, Tehran, Iran
2. Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
Email: haghbin@aut.ac.ir

Objective: Piezoelectric materials are widely used to mimic the electrophysiological nature of the native bone tissue. Several natural and synthetic polymer and ceramic scaffolds with piezoelectric properties can stimulate the proliferation and dif-

ferentiation of stem cells and consequently, improve bone regeneration at the defected site, by reconstructing the local electrical microenvironment of bone. In this study, a poly(L-lactide) (PLLA)-based electrospun scaffold was fabricated for enhancing bone tissue regeneration.
Materials and Methods: Electrospun scaffolds were fabricated using different PLLA/hydroxyapatite ratios. For this purpose, the ceramic component was dispersed in chloroform, and then the polymer component and dimethylformamide solvent were added to the system to be mixed. The electrospinning was carried out with a voltage of 20 kV, a distance of 20 cm between the collector and the needle, and a flow rate of 0.7 ml/h. The nanofibers were characterized using scanning electron microscopy (SEM) and piezoelectric properties were calculated with a homemade piezo-tester.
Results: Electrospun fibers showed a bead-free morphology with a nanometer scale. Increasing the amount of ceramic component in electrospun nanocomposite increases the piezoelectric property, with the maximum piezoelectric value of 0.1456 mV/N.
Conclusion: The fabricated scaffold (PLLA/HA; 80:20) is suitable for bone tissue engineering by virtue of its nanofibrous morphology and piezoelectric property. Furthermore, the presence of hydroxyapatite makes the aforementioned nanocomposite a promising candidate for bone regeneration.
Keywords: Electrospinning, Bone Tissue Engineering, Nanocomposite Fibers, Piezoelectric

Ps-23: Genomic deletion of linc01116 promoter using CRISPR/Cas9 technique and determination of the linc01116 knock-out effect on the breast cancer cell proliferation

Gandomkar G[1, 2*], Rezaei N[2], Dormiani K[2]

1. Department of Biology, Faculty of Science and Technology, ACECR, Institute of Higher Education, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: k_dormiani@royaninstitute.org

Objective: Linc01116 has been shown to increase breast cancer cell proliferation, the severity of malignancy, invasion, and metastasis. Therefore, this LncRNA is being proposed that plays an oncogenic role and also is suggested as a prognostic and therapeutic target in breast cancers. The present study aimed to specifically target the linc01116 promoter sequence in breast cancer cell lines using the CRISPR/Cas9 technique. In the following, the efficiency of this method in the targeted deletion of genomic sequence and thus the inhibition of mentioned LncRNA transcription activity will be examined.
Materials and Methods: First, the expression level of linc01116 in the non-malignant breast epithelial cells (MCF10A), as well as tumor cells (MDM MB231, MCF7, and SKBR3), were evaluated by RT-qPCR. Then, two separate plasmids with mCherry and EGFP reporters were constructed harboring sgRNA coding sequences and Cas9 protein which were subsequently co-transfected by electroporation into the target cells. Finally, transfected cell lines were examined to determine genomic changes, the expression level of linc01116, cell proliferation, and invasion rate.
Results: The results demonstrated that the Linc01116 expression level was significantly increased in breast cancer lines compared with normal breast cells. The accuracy of constructed vectors was confirmed using PCR, sequence analysis, and restriction digestion. Flow cytometry and fluorescent microscope determined the expression of the EGFP and mCherry reporter genes. Also, the efficient deletion of target sequences was revealed by genomic PCR.
Conclusion: Based on the results, growth rate, proliferation, and invasion of breast cancer cells were reduced as a consequence of Linc01116 knockout through the CRISPR/Cas9 system. Thus, targeting this oncogenic lncRNA has a promising advantage in the effective diagnosis and treatment of breast cancer.
Keywords: Breast Cancer, CRISPR/Cas9, Linc01116

Ps-24: Manufacturing of Wharton's Jelly -Derived Mesenchymal Stem Cells (WJ-MSCs) by Enzymatic Cocktail

Ghaderi L[1*], Nouri M[1], Zarrabi M[1, 2]

1. Department of Research and Development, Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: M.Nouri@rsct.ir

Objective: The isolation of Wharton's Jelly -Derived Mesenchymal Stem Cells (WJ-MSCs) from naïve umbilical cord tissue (UC) is particularly important for clinical use; therefore, according to the increasing demand for cellular therapy, it needs to be safe, cost effective, and scaled up. However, current methods to isolate MSCs from WJ yield low amounts of cells with variable proliferation potentials. In this study we successfully designed a new protocol to derivate MSCs by enzymatic approach from fresh samples which presents efficiency and consistency as it allows simultaneous processing of multiple UC samples to produce confluent cultures in a predictable and short timeframe, without impacting on many of the properties that are characteristic of MSCs or potentially therapeutic.
Materials and Methods: UC tissue was cut into smaller pieces. Minced tissue was digested with collagenase I at 37°C and then, centrifuged at 400 g. Supernatant was removed and the enzymatic cocktail (collagenase I and hyaluronidase) added to the pellet for further digestion, and incubated for about 20 minutes. Finally, the enzyme stop solution was added to terminate the incubation period; then, filtrated by cell strainer.
Results: The enzymatic cocktail can be used to isolate large numbers of MSCs in less than 24 hours. All the fresh isolated MSCs had typical fibroblastic morphology, expressed MSC-surface markers (CD73, CD90, CD105), and effectively differentiated into three mesodermal lineages. The cell count per gram of WJ processed by enzymatic method was $4.08 \pm 1.25 \times 10^5$ cells, after 5 days of primary culture (p0); which is significantly on average 1.4 higher when compared with explantation approach for the same WJ, but after 25 days of p0 ($3.3 \pm 0.77 \times 10^5$ cells).
Conclusion: By this protocol, we successfully reduced the required time of primary culture to produce large-amount and high-quality MSCs, which will be suitable for cord tissue banks in the case of providing therapeutic services.
Keywords: Enzymatic Cocktail, Isolation, Mesenchymal Stem Cells, Umbilical Cord Tissue, Wharton's Jelly

Ps-25: A Xeno-Free Protocol for Umbilical Cord Tissue Cryopreservation and Subsequent Mesenchymal Stem Cell Isolation

Ghaderi L[1*], Nouri M[1], Zarrabi M[1, 2]

1. Department of Research and Development, Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: M.Nouri@rsct.ir

Objective: Umbilical cord (UC) tissue, which is once considered as clinical waste, is recognized today a valuable source of mesenchymal stromal cells (MSCs). It has been demonstrated that *in vitro* long-term expansion of cells could induce cellular senescence which is associated with reduction of therapeutic properties. Therefore, it may be prudent to cryopreserve MSCs in the UC format; so that, it would provide access to MSCs from the same donor at any future time point and the cells can be culture expanded again from passage zero (P0) stage. In this study, we introduced an improved protracted cryo-conservation protocol and evaluated the freezing impact on subsequent extracted MSCs in different time intervals, by four cryogenic compositions and two principal isolation approaches: enzymatic digestion and explantation.
Materials and Methods: UCMSCs were isolated from 10 tissue samples. For each fresh UC, a portion was designated for explantation, the second was used for digestion and the remaining third portion was assigned for tissue cryopreservation. Different criteria of fresh and cryopreserved UC derived MSCs including, viability, immunophenotyping profile, proliferative ability, colony-forming potential, migration, and cellular senescence were evaluated and compared.
Results: A xeno-free and sugar containing formulation, with DMSO and autologous plasma, exhibited significantly greater cell yield/ gram of UC at different cryostorage intervals. This advantage was present only for frozen explants after defrosting, while the utilization of enzymatic reaction on cryopreserved samples resulted in significantly fewer viable cells.
Conclusion: The findings of the present study suggest that explant procedure is a safer and reasonable way to isolate MSCs after UC cryopreservation; whereas, enzymatic model may be an optimal strategy for fresh systems.
Keywords: Cryopreservation, Cryogenic Compositions, Isolation Methods, Mesenchymal Stem Cell, Umbilical Cord Tissue

Ps-26: Biological Pathway Alteration at Pediatric Wild Type Acute Myeloid Leukemia and FLT3 Related AML

Ghadimi Nejad Anari Z[1*], Taleahmad S[2]

1. Department of Molecular Genetics, Faculty of 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: s.taleahmad@royan-rc.ac.ir

Objective: FLT3 is one of the significant genes involved in acute myeloid leukemia (AML). It's activation results in apoptosis, proliferation, and differentiation of hematopoietic cells. It has two mutation hotspots which leads to constitutive activation and causing AML. The survival rate for FLT3 related AML is nearly double fold lower than wild type AML.
Materials and Methods: The dataset with GEO accession number GSE17855 has been used, and the data analysis was progressed with R. studio using 50 wild type AML samples and 48 FLT3-related AML. The p value was set on 0.01, resulted in 5000 genes. We Obtained approximately 200 hub genes using StRING and Cytoscape 3.6.0. Once again using StRING and Gephi 0.9.5 the hubs of hub genes and its modules were obtained. After surveying genes on Enrichr, the joint pathways of five databases were obtained and analyzed.
Results: Approximately 80% of pathways with down regulated genes and 91% of pathways with up regulated genes that were involved, were validated by literature review.
Conclusion: The most repeated altered pathways overall were neurodegeneration and brain diseases such as Parkinson and Alzheimer, mRNA processing and chromatin remodeling, gene regulation, apoptosis and cell cycle, proteasome, focal adhesion, estrogen and thyroid hormone, insulin related pathways, breast cancer, oocyte maturation, and ovarian infertility.
Keywords: Acute Myeloid Leukemia, AML, Cancer, FLT3 Related AML, Microarray Analysis

Ps-27: Screening of Different Eryngium Extracts on Beta Cell Regeneration Using Transgenic Zebrafish Model

Hadian M[1*], Ayyari M[2], Tahamtani Y[3, 4], Rezaei M[3, 4]

1. Department of Molecular Genetics, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
3. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: y.tahamtani@royan-rc.ac.ir

Objective: In type 1 diabetes and at the final stages of type 2 diabetes, pancreatic beta cells mass is decreased. One of the proposed approaches for beta cells replacement is endogenous regeneration of these cells. Zebrafish is a powerful model for drug screening studies, because of regenerative capacity in different organs, transparency in larvae stage, and high fecundity. Many studies have been conducted to find the effective compound in regeneration of beta cells that have been led to identification of different signaling pathways and small molecule inducers. Iranian traditional medicine has advised some medical plant for diabetes treatment including Boghnagh (genus Eryngium) which its biologically functional compounds and mechanisms of action needs further investigation.
Materials and Methods: In this study, different species of Boghnagh plant was collected from different regions in Iran and extracts were obtained from root, leave, and aerial parts of this plant. The larvae of transgenic zebrafish Tg(ins:CFP-NTR) at 3 days post fertilization was exposed to metronidazole (MTZ) for ablation of beta cells. After 24 hours and washing of MTZ, the larvae was treated with different concentrations of extracts for 2 days. The effect of treatments on beta cell regeneration was evaluated by imaging of larvae under fluorescent stereomicroscope and measuring the beta cell area by imageJ software.
Results: Results showed that the ethyl acetate extracts from aerial parts gathered from special regions of the country, have significant effects (P value <0.05) in beta cell regeneration in comparison with controls groups.

Conclusion: These findings can led us to further fractionation studies of the extract to find the hit compound/compounds and also for further pre-clinical studies using higher animals of beta cell regeneration.
Keywords: Beta Cells, Diabetes, Eryngium, Regeneration, Zebrafish

Ps-28: Evaluation of The Anti-Cancer Effects of GLI Decoy Oligodeoxynucleotide on Hepatocellular Carcinoma Cells

Hajilou Z[1, 2*], Solhi R[3], Shokoohian B[3], Torabi S[3], Farzaneh Z[2], Keshavarz Alikhani H[3], Vosough M[2, 3]

1. Department of Developmental Biology, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: m.vosough@royan-rc.ac.ir

Objective: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-associated death. The prognosis for patients who are diagnosed with advanced stage HCC is really poor. Recent researches have focused on the identification of novel molecular entities that can be targeted to inhibit oncogenic signals in the carcinogenesis of HCC. Hepatocarcinogenesis involves numerous interlinked factors and processes, including the Sonic hedgehog (Shh) signaling pathway, which participates in the initiation, progression, invasiveness, recurrence, and cancer stem cell maintenance of HCC. In this study, we highlight the importance of glioma-associated oncogene (GLI) transcription factors; the main downstream effectors of the Shh cascade. Therefore, Gli may be a promising target for treatment of tumor cells. Gli-specific decoy oligonucleotides (Gli-decoy ODN) that contain this consensus sequence can inhibit the transcriptional activity of Gli, leading to decrease cancerous phenotypes. The impact of the Gli-decoy ODN was analyzed on the HCC cell line, Huh-7.
Materials and Methods: A Gli-decoy ODN was transfected into Huh-7 HCC cell line *in vitro* by using X-tremeGENE HP DNA Transfection Reagent. The fluorescent microscopy was used to detect the transfection efficiency and the sub-cellular localization of Gli-decoy ODN in Huh-7 cells.
Results: Gli-decoy ODNs treatment showed decreasing cell viability (11.5%). The percentage of cell death was measured by Neutral red uptake assay. The gene expression of downstream targets such as C-myc, Bcl2, SNAIL1 decreased and E-cad significantly increased after treatment of cells with GLI specific decoy ODN because of suppressing Gli. The expression levels of Gli-target genes were identified by quantitative real-time polymerase chain reaction (qRT-PCR).
Conclusion: Gli-decoy ODN significantly suppressed cancerous phenotype in HCC cells. *In vitro* inhibition of the Shh pathway confirms that Shh is essential in maintaining tumor growth, metastasis, and a mesenchymal phenotype, indicating that Gli-decoy ODN may be a potential therapeutic approach for treatment of HCC.
Keywords: Decoy Oligodeoxynucleotide (dODN), GLI Transcription Factor, Hepatocellular Carcinoma, Sonic Hedgehog (Shh) Signaling

Ps-29: Male Germ Cell Localization and Potential Function of Vimentin Cells during Spermatogonial Stem Cells Differentiation Stages

Hashemi Karoii D[1*], Azizi H[2]

1. Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
2. Faculty of Nanobiotechnology, Amol University of Special Modern Technologies, Amol, Iran
Email: h.azizi@ausmt.ac.ir

Objective: Vimentin is an intermediate filament (IF) that is one of the first filaments to be expressed during spermatogenesis. Vimentin has a variety of functions, including cell shape determination, differentiation, cell motility, cell junction maintenance, intracellular trafficking, and helping in the preservation of proper differentiating of germ cell morphology.
Materials and Methods: This study focused at vimentin expression in two groups of spermatogonia: undifferentiated and differentiated. We used immunocytochemistry (ICC), immunohistochemistry (IHC), and Fluidigm real-time polymerase chain reaction (PCR) to evaluate the vimentin expression *in vivo* and *in vitro*.
Results: According to IHC data, strong vimentin expression was found in the center of seminiferous tubules, whereas low expression was seen in the basal membrane. Under *in vitro* conditions, ICC examination of colonies by isolating differentiated spermatogonia revealed positive expression for the vimentin antibody, whereas vimentin expression was negative in the undifferentiated population. Fluidigm real-time PCR analysis revealed that vimentin expression was significantly higher in differentiated spermatogonia than in undifferentiated spermatogonia (P value <0.05). Our findings revealed that vimentin was elevated during the spermatogenesis differentiation phases, demonstrating that vimentin is an intermediate filament with critical involvement in the differentiation stages of testicular germ cells.
Conclusion: These findings promote further research into the spermatogenic process, both *in vitro* and *in vivo*.
Keywords: Germ Cells, Spermatogonia, Stem Cell, Vimentin

Ps-30: Myosin Heavy Chain 7 Plays An Important Role in Myocardial Wall Development

Hesaraki M[1*], Bora U[2], Pahlavan S[1], Mousavi SA[1], Salehi N[3], Barekat M[4], Baharvand H[1], Ozhan G[2], Totonchi M[1, 3]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Izmir Biomedicine and Genome Center (IBG), Dokuz Eylul University Health Campus, Inciralti-Balcova, 35340 Izmir, Izmir, Turkey
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: m.totonchi@royaninstitute.org

Objective: Cardiomyopathy is a pathologic condition which is associated with ventricular wall dysfunction. Left ventricular noncompaction (LVNC) was primarily classified as an inherited

cardiomyopathy. Although several reports have been published about this disease, but the genetic basis is not fully elucidated yet.
Materials and Methods: Using whole-exome sequencing (WES), we investigated a family with several affected members exhibiting LVNC phenotype. Bioinformatics analysis identified a candidate variation which was checked in all family members using Sanger sequencing. The candidate pathogenic variation was examined for expression pattern, protein sequence, and mutant domains in different organisms. In silico prediction of three-dimensional (3D) structure of native and mutant type of candidate variant was done to analyze the effect of mutation on protein structure and function.
Results: We identified a novel heterozygous missense variant (c.1963C>A:p.Leu655Met) in the gene encoding myosin heavy chain 7 (MYH7) with autosomal dominant inheritance. This gene is evolutionary conserved among different organisms. We identified MYH7 as a highly enriched isoform, compared to other types of myosin heavy chains, in skeletal and cardiac muscles. Furthermore, Myh7 was among a few isoforms of Myh in mouse heart that highly expresses from early embryonic to adult stages. In silico predictions showed an altered actin-myosin binding, resulting in weaker binding energy that can cause LVNC.
Conclusion: A novel mutation c.1963C>A:p.Leu655Met in MYH7 with autosomal dominant inheritance was associated with LVNC. Determining the genotype of the gene in all members of this family could help in early diagnosis of the disease and PGD of the next generation.
Keywords: Left Ventricular Noncompaction (LVNC), Myosin Heavy Chain 7 (MYH7), Whole-Exome Sequencing (WES), Protein Modeling

Ps-31: Pectin-Reinforced Electrospun Nanofibers: Fabrication and Characterization of Highly Biocompatible Mats for Wound Healing Applications

Hosseini A*, Kargozar S, Hosseini SJ, Askari VR, Salarinia R, Ebrahimzadeh-Bideskan AR, Tara F, Kermani F, Nazarnezhad S

Department of Medical Biotechnology and Nanotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: kargozarsaeid@gmail.com

Objective: Utilizing naturally occurring substances for improving the biological properties of wound dressings is among the most promising strategies in biomedicine.
Materials and Methods: We here report the successful synthesis a series of electrospun nanofibers reinforced with natural polysaccharide pectin. The composition of the scaffolds was designed as gelatin (GEL))-poly(ε-caprolactone) (PCL)/ poly (vinyl alcohol) (PVA))-Pectin.
Results: The nanofibrous composites exhibited a smooth surface with fibers' diameter ranging between 315 and 431 nm. The incorporation of pectin led to improvements in the nanofibrous scaffolds regarding hydrophilicity (19° after 5 sec), degradability (41.197 ± 4.2%), and water uptake (241.666 ± 58.92 %). Moreover, in the term of mechanical properties, the highest Young's modulus (2.8 ± 0.6 MPa) related to c-PCL/Gel-PVA/Pec electrospun nanofibers compare to other nanofiber mats. From biological perspective, adding pectin to the polymeric substrates resulted in enhanced antibacterial activity of the mats against both Gram-positive and Gram-negative species. On this point, the c-PVA/Pec mats could inhibit the growth of P. aeruginosa up to 57.1% in comparison to the control group. Wile, the inhibitory effect of c-PCL/GEL-PVA/Pec mats on S. aureus and P. aeruginosa were 8.4% and 22.6%, respectively. Furthermore, the PCL/GEL-PVA/Pec electrospun mats exhibited excellent blood and cell compatibility as well as could stimulate cell migration *in vitro*.
Conclusion: In conclusion, pectin can be easily mixed to biopolymers for preparation of three-dimensional (3D) electrospun nanofibers. Regarding their mechanical and biological performance, electrospun composites can be potentially used as wound dressings; especially in cases where infectious wound management is of great importance.
Keywords: Antibacterial Activity, Electrospun Nanofibers, Natural Polymers, Pectin, Wound Healing

Ps-32: A Meta-Analysis of The Effects of Bone Marrow Mononuclear Cell Therapy on Major Adverse Cardiovascular Events Following Acute Myocardial Infarction

Hosseinpour AR*, Attar A

Department of Cardiovascular Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, TAHA Clinical Trial Group, Shiraz, Iran
Email: attar_armin@yahoo.com

Objective: Stem cell therapy following revascularization in patients with acute myocardial infarction (MI) has been proposed as a potential technique for better ventricular function and clinical outcomes. This study aimed to explore the potential effects of bone marrow mononuclear cell (BM-MNC) therapy on major adverse cardiovascular events (MACE).
Materials and Methods: We systematically search digital databases for potential relevant randomized controlled trials reporting the prevalence of adverse events including rehospitalization for heart failure, myocardial reinfarction, cardiac mortality, and the composite of endpoints following transplantation of BM-MNCs in patients with acute MI. We employed Stata software version 13 for generating risk ratio (RR) using the random-effects model.
Results: A total of 23 trials were included after the initial screening of 1540 records for meta-analysis. BM-MNC therapy led to a significant lower risk of rehospitalization (RR=0.57, 95% CI=0.38-0.84), reinfarction (RR=0.58, 95% CI=0.34-0.99), and composite of endpoints (RR=0.64, 95% CI=0.49-84) in the follow-up period. However, no change was observed in the occurrence of cardiac-related mortality (RR=0.72, 95%CI=0.44-1.20) compared to the control arm.
Conclusion: Utilization of BM-MNCs in the setting of acute MI can substantially cause a lower risk of rehospitalization for heart failure and recurrence of MI but it does not change the risk of cardiovascular mortality in the long term.
Keywords: Bone Marrow Mononuclear Cell, Heart Failure, Myocardial Infarction, Stem Cell

Ps-33: Comparing The Effect of Bone Marrow Mononuclear Cells and Mesenchymal Stem Cells on Left Ventricular Function: A Meta-Analysis of Clinical Trials

Hosseinpour AR*, Attar A

Department of Cardiovascular Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, TAHA Clinical Trial Group, Shiraz, Iran
Email: attar_armin@yahoo.com

Objective: Bone marrow mononuclear cells (BM-MNCs) and mesenchymal stem cells (MSCs) are two lines of stem cells that have been used in the setting of acute myocardial infarction (MI) and both types have displayed clinical efficacy by improving left ventricular ejection fraction (LVEF). However, no study has compared these two types of cells directly.
Materials and Methods: In this meta-analysis, we screened through databases (PubMed, Scopus, Embase, and CENTRAL) searching for clinical trials investigating the impact of either BM-MNCs or MSCs on ventricular indices and hospitalization rate for heart failure (HF). We conducted this meta-analysis using Stata software version 13.
Results: In total, 36 trials (26 trials on BM-MNCs and 10 trials on MSCs) were found eligible after applying the inclusion criteria. Both types of cells displayed significant improvement in LVEF in short-term follow-up after transplantation of stem cells when compared to the control group (BM-MNCs: WMD=2.13%, 95% CI=1.23-3.04, p value <0.001; MSCs: WMD=3.71%, 95% CI=2.32-5.09, p value <0.001) but there was no difference between two types of cells regarding LVEF improvement values (p value =0.1). Also, none of the cells decreased the hospitalization rate for HF significantly (BM-MNCs: RR=0.64, 95% CI=0.40-1.02, p value =0.058; MSCs: RR=0.95, 95% CI=0.59-1.51, p value =0.813) and neither kind were superior to another regarding rehospitalization rates (p value =0.26).
Conclusion: The literature lacks relevant trials comparing the efficacy of BM-MNCs and MSCs in patients with acute MI. Although we found that there may not be a more efficacious type of stem cell in the clinical setting, this warrants future trials directly comparing these two kinds of stem cells.
Keywords: Mesenchymal Stem Cell, Bone Marrow Mononuclear Cell, Acute Myocardial Infarction, Stem Cell

Ps-34: hsa-miR-496 and hsa-miR-324 Critical Biomarkers Extracellular Vesicles miRNAs in Astrocyte and Oligodendrocyte Derived Amyotrophic Lateral Sclerosis

Jafari E[1*], Bereimipour A[2], Arabi M[2]

1. Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Departmet of Molecullar Genetics, Faculty of Science and Advanced Technologies in Biology, University of Science and Culture, Tehran Iran
Email: ahmadlta@yahoo.com

Objective: Amyotrophic Lateral Sclerosis (ALS), a degenerative illness that can lead to death, begins with the loss of motor neurons in the brain and spinal cord. Medical researchers are still trying to figure out molecular mechanisms in neurological diseases like ALS. Both astrocytes and oligodendrocyte dysfunction can hasten the progression of the disease in this case.
Materials and Methods: A bioinformatics approach was used to look at the molecular mechanisms and discover important elements between these two cell types in ALS. In this study, we looked at genes, protein products, and miRNAs in astrocytes and oligodendrocytes utilizing integrated and continuous bioinformatics analytics via multiple tools and databases.
Results: Cellular senescence, actin cytoskeleton, and cell cycle signaling pathways were all involved in the findings acquired. When all the information was analyzed, TP53, MDM2, KRAS, PTPRC, and GSK proteins were identified as possible targets of hsa-miR-496-5p, hsa-miR-396-5p, and hsa-miR-4258-3p miRNAs, respectively.
Conclusion: Finally, in this investigation of ALS produced from astrocytes and oligodendrocytes, the four genes had a more robust and better association.
Keywords: Amyotrophic Lateral Sclerosis, Astrocyte, MiRNAs, Oligodendrocyte

Ps-35: Fabrication of 10-HDA / Royal Jelly-Containing Liposomes and The Evaluation of Inflammatory Properties

Javanmardi Isfahani A[1*], Mirahmadi-Zare SZ[2], Saneei N[2], Shafiee N[2]

1. Department of Microbiol Biotechnology, Faculty of Science and Technology, ACECR, Institute of Higher Edication, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: sz.mirahmadizare@royan-rc.ac.ir

Objective: Liposomes are bilayer Nano vesicles which can trap and hold hydrophilic and hydrophobic substances. Similarity to the cell membrane made liposomes useful in drug delivery. This technique is reducing the dose of drugs and also improves their efficiency. The human generation are using bee products for years. Honey is well-known for its effects on the human body. Royal Jelly (RJ), is another bee product that has shown significant antibacterial, anti-inflammatory, and restorative activity. 10-HDA (10-hydroxy-2-decanoic acid) is the active component in RJ. While the composition of the percentage of royal jelly varies according to its origin, bee breed, and storage conditions, 10-HDA has a specific chemical structure.
Materials and Methods: The thin-film hydration method has been chosen for liposome preparation. The shape and structure of prepared liposomes were checked by scanning electron microscopy (SEM) and (TEM). Also, liposome surface functional groups were analyzed by infrared (IR) spectroscopy. Changes in the hydrophilic radius and surface charge of liposomes were investigated using DLS and Zetasizer. Also, the loading efficiency and release rate of the liposomes were evaluated by UV-Vis and fluorescent spectroscopy.
Results: In skin diseases infections, inflammation, and the need for the high permeability of drugs in the skin are important. in this study, (RJ) has been loaded in Nano liposomes for its antibacterial and anti-inflammatory properties.
Conclusion: Based on research and the importance of finding something to replace chemical antibiotics, we expect Royall Jelly or 10-HDA can be useful as an organic antibiotic. We hope our study help to find an organic, non-toxic, and natural substance for replacing chemical antibiotics.
Keywords: Anti-Bacterial, Anti-Inflammatory, 10-HDA, Liposome, Royal Jelly

Ps-36: Fabrication of 10-HDA / Royal Jelly-Containing Liposomes from Soybean Lecithin and Evaluation of Their Anti-Bacterial and Inflammatory Properties

Javanmardi Isfahani A[1*], Mirahmadi-Zare SZ[2]

1. Department of Microbiol Biotechnology, Faculty of Science and Technology, ACECR, Institute of Higher Edication, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: sz.mirahmadizare@royan-rc.ac.ir

Objective: Liposomes are bilayer and spherical structures that can carry a variety of materials therefore widely used in modern drug delivery. For many years so far, the human race has made tremendous use of bee products. Honey is well known for its medicinal and nutritional properties, although Royal jelly attracted attention for its effects on human life. The most noticeable fatty acid in the Royal Jelly structure is labeled 10-HDA (10-hydroxy-2-decanoic acid). Due to its high anti-bacterial and antioxidant activity, widely used in the health and cosmetics industries.
Materials and Methods: We are going to use the thin-layer method for liposome preparation. Materials include soy lecithin, ethanol, chloroform, cholesterol, CTAB, 10-HDA, and Royal Jelly. The next step is to characterize liposomes to analyze toxicity. At last, we will test their anti-bacterial and anti-inflammatory potentials.
Results: In using liposomes as drug carriers, reducing manufacturing costs is serious. Soy lecithin is an organic, non-toxic substance for preparing liposomes. The cost of liposome preparation by using soy lecithin will decrease. Also, we expect to see the anti-bacterial and anti-inflammatory effects from prepared liposomes.
Conclusion: Based on research and the importance of finding something to replace chemical antibiotics, we expect Royall Jelly or 10-HDA can be useful as an organic antibiotic. We hope our study help to find an organic, non-toxic, and natural substance for replacing chemical antibiotics.
Keywords: Anti-Bacterial, Anti-Inflammatory, 10-HDA, Liposome, Royal Jelly

Ps-37: Adipose-Derived Mesenchymal Stem Cells (ASCs) with Shilajit Synergistically Accelerate and Promote Rat Bone Defect

Kangari P[1, 2, 3*], Talaei-Khozani T[2, 7], Razmkhah M[2, 3], Tanideh N[4, 5], Roshangar L[6], Afshari F[1, 2]

1. Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Medical Sciences and Technologies ,Shiraz University of Medical Sciences, Shiraz, Iran
3. Shiraz Institute for Cancer Research, School of Medicine ,Shiraz University of Medical Sciences, Shiraz, Iran
4. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
5. Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
6. Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
7. Tissue Engineering Laboratory, Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Emails: razmkhahm@sums.ac.ir

Objective: Mesenchymal stem cell (MSC) therapy is an amazing revolution in clinical therapy of various skeletal disease such as bone defects. Using crude herbal extracts such as Shilajit was common in bone fracture repair. The purpose of the current study was to discover the synergic effects of ASCs (human adipose derived mesenchymal stem cells) and Shilajit in combination of alginate on the repair of rat tibia bone defect.
Materials and Methods: Seventy-five healthy Sprague Dawley (SD) rats were used in this study. A 7 mm defect was made in right tibia of each rat, and then they were divided into five groups including alginate+Shilajit+ASCs, alginate+ASCs, alginate+Shilajit, alginate, and untreated control groups. Specimens were collected at 2 and 4 weeks after surgery and then bone formation was assayed via radiology, H&E and trichrome Masson staining, and biomechanical tests.
Results: The population doubling time (PDT) test indicated a significant decrease in doubling time of treated ASCs than of the untreated group (P value <0.01). The X-ray photography of tibia specimens showed less repair in the alginate and untreated groups than all other groups at weeks 2 and 4. *In vivo* morphometrically estimations showed predominant synergic effects of alginate+Shilajit+ASCs on increasing the number of osteoblasts, osteocytes, and bone area relative to the alginate+ASCs, alginate+Shilajit, alginate, and untreated groups at week 2 (P value <0.0001). Beside, strength of rat tibia bone showed significant elevation in the alginate+Shilajit+ASCs compare to the alginate+ASCs (P value <0.05), alginate+Shilajit (P value <0.01), alginate, and untreated groups (P value < 0.001 for both). Trichrome Masson staining revealed that collagen deposition was higher in the alginate+Shilajit+ASCs compare to the other groups at week 2.
Conclusion: The combination of ASCs, Shilajit and alginate cause acceleration and promotion of bone tissue regeneration and provide a suitable approach for cell therapy of bone defects.
Keywords: Alginate, Adipose-Derived Mesenchymal Stem Cells (ASCs), Bone Defect, Shilajit, Stem Cell Therapy

Ps-38: Differentiation of Adipose-Derived Mesenchymal Stem Cell (ASC) to Osteoblast in The Presence of Shilajit/ Alginate Combination

Kangari P[1, 2, 3*], Razmkhah M[9, 10], Iraji A[5, 6], Talaei-Khozani T[7, 8], Roshangar L[4]

1. Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
3. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
4. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
5. Stem Cells Technology Researc Center, Shiraz University of Medical Sciences, Shiraz, Iran
6. Central Research Laboratory, Shiraz University of Medical Sciences, Shiraz, Iran
7. Tissue Engineering Laboratory, Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
8. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
9. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
10. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
Email: razmkhahm@sums.ac.ir

Objective: Shilajit, as a herbomineral natural substance, has been most widely used for the remedy of a numerous of illness such as bone defects in traditional folk medicine since hundreds of years ago. The aim of the present study was to explore the effect of Shilajit on the osteogenic differentiation

of human(adipose derived mesenchymal stem cells (ASCs) in two- and three-dimensional cultures.
Materials and Methods: ASCs were isolated and suspended in 1% (w/v) alginate solution with or without Shilajit and seeded into culture plates at a density of 15×10^4 cell per 500µL /well for three-dimensional (3D) cultures. For two-dimensional (2D) cultures, a suspension of ASCs was seeded into culture plates at a density of 15×10^3 cell/well and treated with 500 µg/ml Shilajit. Then, characterization was done using SEM/EDX, ALP (alkaline phosphatase) activity, Alizarin red staining, and Raman confocal microscopy.
Results: According to findings, Shilajit/alginate combination in the presence or absence of osteogenic medium significantly increased ALP activity and mineralization, compared to the 2D matched groups at all time points (P value <0.05). In addition, calcium deposition level was obviously high in Shilajit/ alginate combination with or without osteogenic medium compared to 2D matched groups on days 14 (P value <0.0001), and 21 (P value <0.001and P value <0.01, respectively). Shilajit could also promote osteogenic differentiation of stem cells with or without osteogenic medium in two-dimensional cultures. But Shilajit/alginate combination starts osteogenic differentiation in a short period of time.
Conclusion: As Shilajit accelerates the differentiation of ASCs into the osteoblasts, without changing the physical properties of the alginate hydrogel, this combination may pave the way for more promising remedies considering bone defects.
Keywords: Alginate, Mesenchymal Stem Cell, Osteogenic Differentiation, Shilajit

Ps-39: AICAR and NAM Synergistically Attenuate Senescence-Associated Changes in Mesenchymal Stem Cells: The Interplay of Autophagy and mTORC1

Khorraminejad Shirazi M[1*], Dorvash M[1], Faghihi MA[5], Attar A[2], Sani M[3], Monabati A[6], Talaei-Khozani T[4], Mirzaei M[5]

1. Student research committee ,Shiraz University of Medical Sciences ,Shiraz ,Iran
2. Department of Cardiovascular Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran
4. Tissue Engineering Laboratory, Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
5. Persian BayanGene Research and Training Center, Shiraz University of Medical Sciences, Shiraz, Iran
6. Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran
Email: attar_armin@yahoo.com

Objective: Tissue engineering has yet to reach its ideal goal, i.e. creating profitable off-the-shelf tissues and organs. One of the major challenges of this field that affects the outcome of the cell transplantation is the *in vitro* aging and replicative senescence of the cell source.
Materials and Methods: In the present work, Passage 5 (P5) adipose-derived mesenchymal stem cells (MSCs) were treated with control media, nicotinamide (NAM), AICAR, or the combination of AICAR+NAM till P10. Proliferative capacity, senescence-associated changes, multi-lineage differentiation potential, CDK-inhibitors -P16 and P21-, ROS, and apoptotic markers were compared between cells of P5 and P10. Furthermore, we studied the effects of AICAR and NAM on mTORC1 activity, and autophagy.
Results: Our results showed that MSCs treated with NAM, AICAR, or both demonstrated an increase in proliferation and osteogenic differentiation potential, which was amplified in the group receiving both. AICAR or NAM treatment resulted in reduced expression of β-galactosidase and decreased accumulation of dysfunctional lysosomes. Also, NAM or AICAR+NAM significantly reduced the cellular ROS in aged MSCs. Moreover, AICAR and NAM administration attenuated mTORC1 activity and boosted autophagy.
Conclusion: In general, inhibition of mTORC1 by AICAR and NAM upregulates autophagy, retains MSCs' self-renewal capacity, and delays senescence of MSCs after prolonged *in vitro* culture. Furthermore, our findings showed that concomitant use of AICAR and NAM shows a synergistic effect on the attenuation of cellular senescence. Our findings provide a viable option to slow down the *in vitro* aging of the stem cells and conquer aging as a limiting factor in tissue engineering.
Keywords: Aging, Autophagy, mTOR, Senescence

Ps-40: PIWIL2 Promotes The Growth and Colonization Rate of Colorectal Cancer Cells

Kishani Farahani R*, Soleimanpour-Lichaei HR

Department of Stem Cells and Regenerative Medicine, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
Email: hrs@nigeb.ac.ir

Objective: Unlimited proliferation caused by unbalanced cell cycle progression and apoptosis is responsible for tumor initiation, development and metastasis. In this research, the role of PIWIL2 overexpression on this imbalance was investigated in SW480 cells as a colorectal cancer cell (CRC) line.
Materials and Methods: PIWIL2-overexpressing and control SW480 cells were established by transfecting PCDNA3-PIWIL2 and PCDNA3-empty vectors into SW480 cell line, respectively via electroporation. The relationship between the PIWIL2 gene overexpression on the rate of proliferation, cell cycle progression and apoptosis genes were determined by real-time polymerase chain reaction (PCR), MTT assay, Doubling Time assay and colony formation.
Results: A significant increase in StAT3, BCL2-L1, BCL2-L2, cyclin D1 and Ki-67 genes expression were found in SW480-PIWIL2 compared to SW480-control cells. Colony formation analysis showed a ~4-fold increase in the number of colonies (P value <0.001). Furthermore, the results of Doubling Time assay and MTT indicated that PIWIL2 overexpression induced time-dependent effects on proliferation rate.
Conclusion: In this study our data indicated that PIWIL2 overexpression promoted the proliferative capacity of CRC cells as well as cell colonization and inhibition of apoptosis in these cells. These data revealed the importance of PIWIL2 gene as a valuable oncogenic biomarker for the purpose of cancer diagnosis and targeted therapies.
Keywords: Apoptosis, Colorectal Cancer Cell (CRC), PIWIL2, Proliferation

Ps-41: CRISPR/Cas9 Highly Popular Vectors Preparation; Recommendations and Points

Kolahdouz Mohammadi M[1, 2*], Pahlavan S[2], Ostadi L[2], Sotoo-

dehnejad Nematalahi F[1], Totonchi M[2]

1. Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad 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: m.totonchi@royaninstitute.org

Objective: Genome editing is currently used as an efficient and accurate method in biological and medical studies. Among the wide range of genome editing techniques, clustered regularly interspaced short palindromic repeats (CRISPR) is one of the simplest yet promising method. The CRISPR system consists of two key components; an endonuclease enzyme called Cas9 and guide RNA (gRNA), ensuring that the Cas9 enzyme cuts at the right point in the genome. Although CRISPR genome editing is one of the useful methods to modify the genome, there are still multiple challenges in the technique's steps. In this presentation, we tried to define these challenges and recommend appropriate solutions.
Materials and Methods: sgRNAs were designed and ordered accordingly based on the target site and vector of interest. Cloning procedures were performed and confirmed by sequencing as a gold standard. The list of available sgRNAs for the LAMP2B gene was conducted based on available databases by analyzing and comparing the specificity and functionality as two main characteristics.
Results: In this study, we tried to transfer our experiences regarding the main challenges in the process of preparing CRISPR/Cas9 popular vectors (pX330/pX459). A complete list of sgRNAs for an autophagy gene is also prepared as an example to clarify the sgRNA design procedures.
Conclusion: This presentation tried to depict problems that may be encountered during sgRNA design and plasmid preparation, followed by appropriate recommendations.
Keywords: CRISPR/Cas9, Genome Editing, sgRNA, pX330/pX459, Plasmid

Ps-42: AMPK Activation Can Promote Cardiac Differentiation by Stimulating Autophagy Pathway

Kolahdouz Mohammadi M[1, 2*], Pahlavan S[2], Salehi N[3], Sotoodehnejad Nematalahi F[1], Totonchi M[2]

1. Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad 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
3. School of Biological Science, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran
Email: m.totonchi@royaninstitute.org

Objective: It has been shown that autophagy, an essential catabolic process for survival under stress, contributes to differentiation into various types of cells such as cardiomyocytes. Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) is an energy-sensing protein kinase involved in the regulation of autophagy. It has been demonstrated that AMPK plays a role in many other cellular processes. Due to its involvement in multiple cellular processes, AMPK can influence the survival and health of cardiomyocytes. In this study, the time-dependent effects of an activator of AMPK (Metformin) and an autophagy inhibitor (Hydroxychloroquine) on human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) differentiation were investigated.
Materials and Methods: During cardiomyocyte differentiation, Metformin and Hydroxychloroquine were used to induce and inhibit autophagy, respectively. Days 0, 2, 4, and 13 were examined for the expression patterns of the autophagy genes.
Results: During cardiomyocyte differentiation, autophagy was upregulated, and it was not affected by autophagy inhibitor/inducer. AMPK activation also increased the expression of cardiomyocyte-specific markers in hPSC-CMs. Also, autophagy inhibition by targeting autophagosome-lysosome fusion impaired cardiomyocyte differentiation.
Conclusion: In conclusion, AMPK might be a promising target for regulating cardiomyocyte generation by *in vitro* differentiation of pluripotent stem cells, due to the importance of autophagy in this cellular process.
Keywords: Autophagy, Adenosine 5′-monophosphate-activated protein kinase (AMPK), Cardiomyocyte Differentiation, Human Pluripotent Stem Cell (hPSC)

Ps-43: Cell Alignment and Neurite Elongation during Motor Neuron Differentiation with Epothilone B in The Presence of Neurite Outgrowth Inhibitor-A

Mahmoodi N[1*], Ai J[2], Ebrahimi-Barough S[2], Hassannejad Z[3], Hasanzadeh E[4], Nekounam H[5], Rahimi-Movaghar V[1]

1. Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Tissue Engineering, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Pediatric Urology and Regenerative Medicine Research Center, Tissue, Cell and Gene Research Institute, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Tissue Engineering & Regenerative Medicine, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran
5. Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: n.mahmoodi85@gmail.com

Objective: Neuronal cell death and axonal degeneration are common pathophysiological occurrences after spinal cord injury (SCI). Improving stem cell differentiation to replace lost and damaged cells after SCI might be a potential approach. Many investigations are now underway to establish procedures for motor neuron differentiation in order to regenerate cells that are similar to the lost endogenous ones as a potential cure for SCI and neurodegenerative illnesses. The goal of this work was to see how epothilone B (EpoB) affected the differentiation of human endometrial stem cells (hEnSCs) into motor neuron (MN) like cells in the presence of neurite outgrowth inhibitor A (Nogo-A).
Materials and Methods: hEnSCs were differentiated into motor neuron-like cells in three steps (pre-induction, induction, and maturation). The Epo B differentiation group was treated with 10 nM EpoB (added in steps 2 and 3). The cells were differentiated in the presence of NogoA (400 ng/mL) to simulate the *in vivo* inhibitory environment. Cell alignment and neurite elongation were observed and measured using an inverted phase-contrast microscope. To reveal the morphology of cells,

they were stained for β-III-tubulin marker by immunofluorescent (IF).
Results: The neurite length of the EpoB group significantly (P < 0.001) increased in comparison with the control group. IF images indicated the stability of neuronal morphology, great cell alignment, and neurite elongation in the EpoB group in the presence of Nogo-A.
Conclusion: Despite the presence of the Nogo-A inhibitor, this study showed EpoB as a booster for neurite elongation and a notable cell alignment organizer during MN differentiation.
Keywords: Cell Alignment, Differentiation, Epothilone B (EpoB), Neurite Elongation, Neurite Outgrowth Inhibitor A (Nogo-A)

Ps-44: Time Depending of Adding Valproate Sodium Improves Motor Neuron-Like Cell Differentiation of Human Endometrial Stem Cells

Mahmoodi N[1*], Ai J[2], Ebrahimi-Barough S[2], Hassannejad Z[3], Hasanzadeh E[4], Nekounam H[5], Rahimi-Movaghar V[1]

1. Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Tissue Engineering, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Pediatric Urology and Regenerative Medicine Research Center, Tissue, Cell and Gene Research Institute, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Tissue Engineering & Regenerative Medicine, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Sari, Iran
5. Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: n.mahmoodi85@gmail.com

Objective: Substituting differentiated stem cells for lost and damaged cells is one of the proposed therapeutic options for spinal cord injury (SCI). Human endometrial stem cells (hEnSCs) are a potential source of mesenchymal stem cells that can differentiate into various neural cell types. Many researchers are developing protocols for motor neuron differentiation to regenerate cells that match the lost endogenous ones as a possible cure for SCI and neurodegenerative illnesses. Valproate sodium (VAS) is a small molecule with anti-inflammatory and neuroprotective features and has the ability to pass the blood-brain barrier. Our goal was to study the effect of VAS on improving motor neuron differentiation of human endometrial stem cells.
Materials and Methods: hEnSCs were extracted via collagenase type I and were authenticated at passage three by flow cytometry. The motor neuron differentiation protocol contained three phases: 1. pre-induction phase (24 hours), 2. induction phase (first week), and 3. survival phase (second week). The VAS (100 µg/mL) was added from phase 2 or phase 3 of differentiation. Immunofluorescence (IF) was used to assess the expression of neurofilament heavy polypeptide (NF-H) and synaptophysin (SYP) after 15 days.
Results: The IF results revealed NF-H and SYP high protein expression (two important neural-specific markers) in the differentiation group, which VAS was added in the third phase (second week) of differentiation. In contrast, the other differentiation group (VAS was added in phase 2) did not show NF-H and SYP expression.
Conclusion: This study points out the importance of time-dependent adding of VAS to improve motor neuron-like cell differentiation of hEnSCs.
Keywords: Human Endometrial Stem Cells (hEnSCs), Neurofilament Heavy Polypeptide (NF-H), Motor Neuron, Synaptophysin (SYP), Valproate Sodium

Ps-45: The Efficacy of Human Umbilical Cord Mesenchymal Stem Cells on Wound Healing and Insulin-Like Growth Factor Expression; An *In Vivo* Study

Malekinejad Z[1*], Safavi SE[1], Barati AR[1], Yosefzadeh F[2]

1. Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Biotechnology Research Center, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Email: safavi.s.e@gmail.com

Objective: Stem cell-based regenerative medicine tries to find novel approaches to treating severe medical disorders including wound. The insulin-like growth factor (IGF) family contributes efficiently to growth, cell proliferation and differentiation. Also hUC-MSCs (human umbilical cord mesenchymal stem cells) play important role in regenerative medicine and they can release insulin-like growth factors.
Materials and Methods: In this examination, 20 male Wistar rats (250 ± 20g) were randomly divided into two groups after wound creation on the dorsal side of the animals. Group I was the control group and normal saline were injected into the peripheral area of the wound intradermal. The second group (group II) was the human umbilical cord hUC-MSCs CM (human umbilical cord mesenchymal stem cells conditioned medium) treatment group which 1cc Wharton´s jelly-derived hUC-MSCs CM was injected in the peripheral area of the wound intradermally. The wound healing rate was measured and marked on the 0th, 5th, 10th and 14th days of the process. Subsequently, all animals were euthanized and the wound samples were stored for complementary assays. The expression level of IGF-1 and IGF-2 were assayed by real-time polymerase chain reaction (PCR).
Results: The results from the measurement of the wounds surfaces showed that the treatment was significantly effective for wound healing in comparison to the control group (P value <0.05). Also the expression level of IGF-1 and IGF-2 was significantly increased in compare with the other group (P value <0.05 and P value <0.005).
Conclusion: HUC-MSCs conditioned medium therapy increased the expression of IGF-1 and IGF-2 in the wound samples and it improved wound healing.
Keywords: Insulin-Like Growth Factor, Mesenchymal Stem Cells, Regenerative Medicine, Wound

Ps-46: Human Umblical Cord Mesenchymal Stem Cells Effects on Wound Healing and Transforming Growth Factor B Overexpression: An *In Vivo* Study

Malekinejad Z[*], Safavi SE, Barati AR

Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Email: safavi.s.e@gmail.com

Objective: Delay in wound healing gives severe health and economic challenges to patients and public health especially

after surgeries. MSCs (mesenchymal stem cells) as multipotent non-hematopoietic progenitors can play a crucial role in tissue regeneration and contribute to wound healing by growth factors secretion.
Materials and Methods: In this study, 20 male Wistar rats (250 ± 20 g) after establishing a square wound on the dorsal side were randomly divided into two groups: I. control group, II. hUC-MSCs CM (human umbilical cord mesenchymal stem cells conditioned medium) treatment group. In the treatment group, 1 cc of Wharton´s jelly-derived hUC-MSCs conditioned medium was injected intradermally into the peripheral area of the wound. The rate of wound healing was measured on the 0th, 5th, 10th and 14th days and the wound area was marked. All animals were euthanized 14 days after the injection and the skin samples were removed and frozen immediately for complementary assessments. The expression of TGFβ-1 and TGFβ-2 (transforming growth factor) were assayed by real-time polymerase chain reaction (PCR).
Results: The results showed that the expression of TGFβ-1 was significantly increased in comparison with the control group (P value <0.05). Although the expression of TGFβ-2 was increased in comparison to the control group but it was not significant. Also, the assessment of the healing of the wound area showed that the treatment was remarkably effective in wounds healing (P value <0.05).
Conclusion: Human umbilical cord mesenchymal stem cells conditioned medium have an effective contribution in the wound amelioration and it can increase the level of TGFβ-1 expression.
Keywords: Regenerative Medicine, Stem Cells, TGF-Beta Superfamily Proteins, Wound

Ps-47: Combination of NK Cell Therapy and Telomerase Inhibition Increases Breast Cancer Stem Cells Apoptosis

Mazloumi Z[1*], Rafat A[2], Dizaji Asl Kh[2], Nozad Charoudeh H[3]

1. Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
3. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: zb.mazloumi@gmail.com

Objective: Breast cancer stem cells are resistance to conventional therapy. NK cell therapy has not been satisfactory due to immunosuppressive of tumor microenvironment in breast cancer. hTERT as tumor-associated antigens (TAAs) is overexpressed in 85% of tumors and has a role in self-renewal, proliferation of the CSC, immortality, and tumorigenesis. Thus, in this study, we inhibited telomerase and evaluated the NK cell cytotoxicity and apoptosis of Breast cancer stem cells.
Materials and Methods: MDA-MB-231cells were treated with IC50 level of BIBR1532. After 24 hours, NK cells were added to the treated cells and incubated for 5 hours. Then, NK cell cytotoxicity, Annexin V/7AAD experiments, and expression of apoptosis related genes were analyzed.
Results: Our results showed that telomerase inhibition increases apoptosis in breast cancer stem cells.
Conclusion: BIBR1532 inhibit telomerase and sensitize breast cancer stem cells to NK cell therapy. Therefore, Telomerase inhibition can be beneficial in the proper function of NK cells.
Keywords: Breast Cancer Stem Cells, NK Cell Therapy, Telomerase

Ps-48: All-Trans Retinoic Acid Enhances Cytotoxicity of CIK Cells Against Hepatocellular Carcinoma

Minaei N[1, 2, 3*], Torabi Sh[1, 2, 3], Piryaei A[4, 5], Ebrahimi M[3], Vosough M[2, 3, 6]

1. Department of Applied Cell Sciences, Faculty of Basic Sciences and Advanced Medical Technologies, Royan Institute, ACECR, Tehran, Iran
2. Department of Regenerative Medicine, 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 Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
6. Experimental Cancer Medicine, Institution for Laboratory Medicine, Karolinska Institute and Karolinska University Hospital-Huddinge, Stockholm, Sweden
Email: masvos@royaninstitute.org

Objective: To determine the increased sensitivity of pretreated hepatocellular carcinoma cells with all-trans retinoic acid (ATRA) to enhance the cytotoxicity of cytokine-induced killer cells (CIKs), we evaluated their effects, alone and in combination, on HepG2 cell line.
Materials and Methods: ATRA is currently used as a potential chemo-preventive agent because of its anti-proliferative, pro-apoptotic and antioxidant properties. This study investigated the effects of ATRA at different concentrations on the proliferation and the expression of MHC class I chain-related protein A and B (MICA and MICB) on HepG2 cell line. Moreover, the anti-tumor activity of CIK cells on the pretreated HCC cell line with ATRA analyzed as well. Peripheral blood mononuclear cells (PBMC) from healthy donor were incubated *in vitro* and induced into CIK cells in the presence of various cytokines. The phenotype, characterization and cytotoxicity of CIK cells were identified by flow cytometry analysis. The effect of ATRA on the proliferation and expression of MICA/B were examined by MTS assay and quantitative RT-PCR, respectively.
Results: ATRA significantly inhibited the cell growth and increased the expression of MICA/B on HepG2 cells in a dose-dependent manner. In addition, CIK cells could eradicated cancerous cells through direct interaction between NKG2D receptor and MICA/B ligand, which are expressed on the surface of CIK cells and on the surface of HCC cell lines, respectively. The combined application of two approaches can synergistically boost cytotoxicity of them through upregulated expression of MICA and MICB on the HepG2 after ATRA treatment.
Conclusion: These results suggest that combination of ATRA and CIK could be a potentially novel treatment protocol for hepatocellular carcinoma.
Keywords: All-Trans Retinoic Acid, Cytokine-Induced Killer Cells, Hepatocellular Carcinoma, Immunotherapy

Ps-49: Spinal Cord Injury Affects Gene Expression of Transmembrane Proteins in Tissue and Extracellular Vesicle Release in Blood: In Silico and *In Vivo* Analysis

Mirzaalikhan Y[1, 2*], Shekari F[2], Kiani S[2]

1. Department of Developmental Biology, Faculty of 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: s.kiani@royan-ac.ec.ir

Objective: Spinal cord injury (SCI) can disrupt membrane transmission by affecting channels or neurotransmitter release. This study aimed to explore gene expression changes underlying SCI in rats by bioinformatics approaches and confirm in RNA and protein levels.
Materials and Methods: The differentially expressed genes (DEGs) in acute and subacute SCI were screened based on GSE464, GSE45006, GSE46988, GSE2599 microarray data downloaded from Gene Expression Omnibus. Transmembrane proteins of DEGs were recognized using the Uniprot and transmembrane helices prediction (TMHMM) methods. Model of SCI was established through a weight dropping procedure in rat. To confirm SCI model hematoxylin and eosin (H&E) staining were performed. Total mRNA was extracted from spinal cord tissues. The RNA expression profile of some of the significantly changed genes in pervious part has been confirmed by real-time polymerase chain reaction (RT-PCR). Blood was collected from rats before sacrificing. EVs are isolated by high-speed centrifugation (20 Kg) from plasma. For assessment of protein expression of CD9, CD63 and Cxcr4 proteins western blotting were used.
Results: Based on bioinformatics analysis, we found a set of common genes in both phases that encode transmembrane proteins. Kcna1, Scn1a, Grm1, Cnr1 and Nrg1 are downregulated; and Olr1, Enpp3, Cd63, Ptprc, Cd53, Adcy4 Adcy4, and Cxcr4 are upregulated. H&E demonstrated tissue damage as well as neuronal degeneration. Following by RT-PCR for tissue, we showed significant upregulation in Grm1, Nrg1, CD63, Enpp3, and Cxcr4 between the acute and control groups and downregulation in Enpp3 between acute and subacute groups. Considering CD63 as an extracellular vesicle marker, we examined the protein expression of CD9 and CD63 plasma derived-EV. CD9 has significant expression between acute and control groups. We also demonstrate no significant CD63 and Cxcr4 expression between groups.
Conclusion: Our results provide novel insight into the relationship between membrane protein expression and SCI. Also, results show that SCI affected EV release in the blood which can help enlarge strategies to enhance recovery following SCI.
Keywords: Bioinformatics, Membrane Proteins, Spinal Cord Injury

Ps-50: Design and Fabrication of Gene Constructs to Generate A Transgenic Zebrafish Model to Study Pancreatic Progenitor Cells during Beta Cell Ablation

Moayedi M[1*], Angaji AH[1], Rezaei M[2, 3], Tahamtani Y[2, 3]

1. Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Karaj, Iran
2. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, 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
Emails: y.tahamtani@royan-rc.ac.irr

Objective: Zebrafish are widely used in beta-cell regenerative research because of its high regeneration capacity, easy genomic manipulation, and potential for high throughput drug screening. Pancreatic duodenal homeobox1 (PDX1) is essential for the development of pancreatic exocrine and endocrine cells including glucose-responsive (insulin-producing) beta cells. Pdx1 mutant zebrafish have the key diabetic features of reduced beta cells, decreased insulin, and elevated glucose. Therefore, the pdx1 transgenic zebrafish is an essential model for diabetes research.
Materials and Methods: In this study, Pdx1:GFP construct was designed using the TOL2 transposase construct to generate transgenic zebrafish (Tg(pdx: GFP)) model. Because the regulatory region of the pdx1 gene isn't defined we design two pairs of primers to amplify 2 kb and 6 kb 5′-flanking sequence upstream of the ATG start codon in zebrafish pdx1 gene and restriction site of SalI enzyme added to ends of pieces. High fidelity polymerase was used to reduce the mismatch in the amplification of these polymerase chain reaction (PCR) products.
Results: Amplified products inserted upstream of GFP using SalI enzyme and recombinant plasmids were confirmed by colony PCR and digestion, and proper fragment sizes were obtained in both tests. This vector could be used for the generation of transgenic zebrafish Tg(pdx1:GFP) by co-injection with transposase mRNA in one-cell stage zebrafish embryos.
Conclusion: This transgenic line could be used for further drug screening experiments and because of pdx1 expression in endocrine progenitor cells this model is useful for the study of neogenesis mechanism in beta cell regeneration research.
Keywords: Beta Cell, Diabetes, Tol2, Transposase, Zebrafish

Ps-51: Minicircle System as An Innovative Approach for Generation of Functional CAR T-Cell

Moghimi Khorasgani A*, Rezaei N, Kiani-Isfahani A, Dormiani K

Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: k_dormiani@royaninstitute.org

Objective: Chimeric antigen receptors (CAR) are receptors that gives T-cells a new ability to target a specific cancer antigen. It has been shown that this strategy is an efficient and safe approach for treatment of hematological and some solid cancers. On the other hand, the minicircle as a nonviral vector has the ability to high level expression of the transgene without adverse effects compared to the viral vectors. In minicircle, prokaryotic sequences are deleted from the parental plasmid and the vector is a smaller in size with a long duration of expression, and safer due to the absence of bacterial sequences. These characteristics have made the minicircle as a promising vector for biomedical applications. In the present study, we applied minicircle as a safe vector to generate specific and functional CAR T-cells.
Materials and Methods: In this study, a coding sequence of the third generation of CAR was constructed, and cloned into a replicative minicircle. This CAR encoding minicircle (CAR-MC) was introduced into T-cells from peripheral blood by electroporation. Then, resulted CAR T-cells were co-cultured with the cancer cells with the specific antigen. Functionality of CAR T-cells were tested *in vitro*. Generated.
Results: The results showed that CAR T-cells produced by CAR-MC, specifically targeted the cancer cells with strong cytotoxic effects compared to negative cells. Additionally, these CAR T-cells also exhibited the ability to secret a signifi-

cantly higher level of inflammatory cytokines compared to the control T-cells.
Conclusion: Overall, the present study showed that the CAR-MC has the capability of improving antitumor activity of the T-cells. In fact, minicircle encoding a functional CAR receptor enabled the T-cells to exclusively target and destroy the malignant cells.
Keywords: Cancer Immunotherapy, CAR T-Cell, CD19, Minicircle

Ps-52: Mesenchymal Stem Cell Tracking Using Natural Based-Nanostructures: A Novel and Non-Invasive Technique for Tissue Engineering

Mohseni M[1*], Mehravi B[2]

1. Stem Cell and Regenerative Medicine Research Center, Iran University of Medical Sciences, Tehran, Iran

2. Department of Medical Nanotechnology, Faculty of Medical Nanotechnologies, Iran University of Medical Sciences, Tehran, Iran

Email: mehravi.b@iums.ac.ir

Objective: In the novel technologies based on tissue engineering for regenerative medicine, determining the stem cells' fate is essential. In this regard, non-invasive tracking of stem cells using natural-based nanostructures would be a promising approach.
Materials and Methods: In this study, chitosan-alginate nanoparticles containing Gd3+ were synthesized using the pregelation method for mesenchymal stem cell tracking using the MRI technique. Characterization methods of SEM, FTIR, TEM, and DLS were utilized for nanoprobe evaluations and stem cell tracking was visualized using an *in vivo* study. For *in vivo* analysis, 5×10^7 labeled cells per pellet with a volume of 0.1 mL (subcutaneous) were injected into the dorsal flank of a male BALB/c mouse.
Results: Results demonstrate that the spherical shape of nanoparticles with the approximate size of 80 nm was synthesized. ICP result shows the Gd3+ concentration of 1958.33 ppm. The values of r1 and r2 in the phantom were 12.8 and 18.754 mM−1s−1 and the r1 and r2 values in cell 7.56 and 22.162 mM−1s−1 were reported. The r1 relaxivity for the nanoparticles in the phantom was 12.8 mM−1s−1 per Gd3+ ion, which is 3.5 times larger than that for Dotarem® (r1 -3.6 mM−1s−1 per Gd3+ ion) and the r1 relaxivity for the nanoparticles in stem cells was 7.56 mM−1s−1 per Gd3+ ion, which is 2.16 times larger than that for Doratem®. The significantly signal enhancement in t2-weighted in *in vivo* images was observed in labeled nanoparticles.
Conclusion: Our results reveal that the aforementioned biocompatible nanoparticles have a high potential for mesenchymal stem cell tracking for tissue engineering applications.
Keywords: Nanoparticles, Stem Cell, Tracking

Ps-53: Investigating The Anticancer Activity of Biosynthesized Cobalt Oxide Nanoparticles *In Vitro*

Momen Eslamiehei F[1*], Moghadam Matin M[1, 2, 3], Mashreghi M[2, 3]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

2. Stem Cell and Regenerative Medicine Research Group, Academic Center for Education, Culture and Research (ACECR)-Khorasan Razavi, Mashhad, Iran

3. Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

Email: matin@um.ac.ir

Objective: In recent decades, nanotechnology has been used for cancer diagnosis at early stages, and also designing drug delivery systems by targeting the nano-drug combination to cancer cells, and thus reducing the cytotoxicity to normal cells. Cobalt oxide is one of the most potent natural anticancer agents which has various applications in medicine, such as drug delivery systems, toxicity to cancer cells, and antibacterial properties. Cytotoxicity of cobalt oxide nanoparticles (Co3O4NPs) can be studied on various cell lines.
Materials and Methods: In this study, we first produced Co3O4 NPs using luminescence bacteria and then characterized their properties and investigated their anticancer activity on colorectal cancer cells. The nanoparticles were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier transform-infra red spectroscopy (FTIR) analysis.
Results: The XRD results illustrated the success in the synthesis of Co3O4NPs. The results of SEM and TEM indicated that the biologically synthesized cobalt oxide nanoparticles are spherical and cubic in shape with about 70 nm diameter. The successful biosynthesis of cobalt oxide nanoparticles, was verified by the presence of amine groups as shown in FTIR analysis. The cytotoxicity study exhibited a time and dose dependent effect against CT26 colorectal cancer cells as shown by MTT assay. The half maximal inhibitory concentration (IC50) was found to be 60 µg/ml after 48 hours. The cytotoxic properties of Co3O4NPs were also studied on mouse embryonic fibroblast cells (NIH3T3) as a normal cell line, where the results indicated lower toxicity of the NPs compared to CT26 cancerous cells.
Conclusion: Based on our results, the biologically synthesized Co3O4NPs were found to specifically decrease the viability of CT26 cancerous cells *in vitro* and thus they exhibit multifunctional properties.
Keywords: Anticancer Activity, Biosynthesis Cobalt Oxide Nanoparticles, CT26 Cell Line, MTT assay

Ps-54: Lactobacillus Casei Supernatant Effect on PANC-1 Cell Viability

Moradi Sh[1*], Karamali F[2], Moradi R[2], Hajian M[2]

1. Department of Microbiol Biotechnology, Faculty of Science and Technology, ACECR, Institute of Higher Edication, Isfahan, Iran

2. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Email: m.hajian@royan-rc.ac.ir

Objective: Probiotics are nonpathogen lactic acid bacteria, which are important in the treatment of digestive disorders and cancers. Probiotics' importance in cancer therapy is related to their effect on the apoptosis pathway and many studies have shown their anti-tumor effects *in vitro* and *in vivo*. According to the world health organization, cancer is the second cause of death in the world and pancreas cancer is the 12th most common cancer. probiotics and their extractions, exopolysaccharides, and peptidoglycans have inhibitory effects on pancreas cancer. Therefore, the study of probiotics' effects on cancer and finding unique treatments has received much attention. In this study, the potential effect of lactobacillus casei on PANC-1 cell viability in comparison to Fibroblast cells has been investigated.

Materials and Methods: PANC-1 and Fibroblast cells were treated with Different concentrations of Lactobacillus casei supernatants (5, 10, 20, and 40 %) for 24 hours and cell viability with MTS assay has been tested.
Results: Our findings showed that Lactobacillus casei supernatant has significantly decreased PANC-1 cells viability and had no significant effects on fibroblast cells. PANC-1 cells viability for 5, 10, 20 and 40 percent of supernatant concentration was 93, 82.33, 61.33 and 45.33%, respectively.
Conclusion: According to the literature review, Lactobacillus casei as a probiotic has anti-cancer effects. Our findings showed that cell viability was significantly decreased in 10, 20, and 40% of Lactobacillus Casei supernatant and it seems that the cell death effect of its supernatant is dose-dependent.
Keywords: Cancer, Lactobacillus, Pancreas, Probiotic

Ps-55: ZIF8-Modified Polypropylene as A Bioactive GBR Membrane: An *In Vivo* Study

Mousavi SJ[1, 2*], Ejeian F[2], Razmjou A[1], Nasr-Esfahani MH[2]

1. Department of Biotechnology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: a.razmjou@ast.ui.ac.ir

Objective: Guided bone regeneration (GBR) is a modern strategy for treatment of periodontal disease. As the major component of the GBR process, the use of barrier membranes prevents soft tissue ingrowth into the defect cavity and provides sufficient space for new bone formation. Beside this barrier role, an ideal GBR membrane needs to have osteo-inductive potential. In line with our previous reports on remarkable *in vitro* osteogenic capacity of zeolitic imidazolate framework 8 (ZIF8) thin layer, *in vivo* bone regeneration was evaluated in a rat calvarium defect model using a ZIF8-modified polypropylene membrane.
Materials and Methods: Mechanical characterization of the membrane revealed reasonable Young modules (35.35 MPa) and flexibility after ZIF8 coating, which were in the range of commercial GBR membranes. Two symmetrical 5-mm defects were created in the calvaria of 8-10-week-old male Wistar rats. One defect in each animal was randomly covered with pristine PP or ZIF8-modified membranes (N=7 per group), while the other defect left untreated as control. Eight weeks post-surgery, animals were sacrificed and bone formation was assessed by H & E staining and immunohistological analysis.
Results: The results clearly verified the barrier function of both PP and PP-ZIF8 membranes in comparison to the uncovered control defects. Furthermore, some marginal bone formation was observed in control and PP groups, while the defect area was almost filled with new mature bone through the use of PP-ZIF8 membranes.
Conclusion: Getting all together, our findings support the feasibility, safety and effectiveness of PP-ZIF8 membrane as a promising new approach for improving GBR outcomes, without using biological components.
Keywords: Guided Bone Regeneration (GBR), GBR Membrane, Zeolitic Imidazolate Framework 8 (ZIF8), Thin Layer

Ps-56: The Effectiveness of Cord Blood Mononuclear Cells Intrathecal Injection on The Developmental Functions of Patients with Spastic Cerebral Palsy; Phase 1 Clinical Trial

Nouri M[1*], Zarrabi M[1, 2], Majmaa A[3], Ghahvechi Akbari M[4], Dehghan E[5], Abroun S[6], Vosough M[2], Samimi S[5], Hamidieh AA[7], Masoomi S[8], Gholami Nejad M[5], Ashrafi MR[8]

1. Department of Research and Development, Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
2. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Pediatrics Center of Excellence, Pediatric Intensive Unit, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
4. Physical Medicine and Rehabilitation Department, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
5. Department of Transplantation, Royan Stem Cell Technology Company, Tehran, Iran
6. Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
7. Pediatrics Center of Excellence, Pediatric Hematology Unit, Department of Oncology and Stem Cell Transplantation, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
8. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
9. Pediatrics Center of Excellence, Department of Pediatric Neurology, Children's Medical Center, Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran
Email: ashrafim@tums.ac.ir

Objective: Cerebral palsy (CP) is the most common physical disability of children that results from non-progressive brain injuries. The current multi-center, randomized, double-blind study was conducted among children with cerebral palsy to assess the safety and efficacy of umbilical cord blood mononuclear cell (UCB-MNC).
Materials and Methods: 72 children with spastic cerebral palsy aged 4 to 14 years were included in two of the experimental or control groups. UCB-MNC cells group were injected at 5 × 106 per kg of child weight, Intrahecally. Primary and secondary outcomes were evaluated at 1, 3, 6, and 12 months after the intervention.
Results: The mean GMFM-66 scores increased in experimental group; compared to baseline (+9.62; 95%CI: 6.75, 12.49) and control arm (β: 7.10; 95%CI: 2.08, 12.76; Cohen's: 0.62) and mean MAS reduced in individuals treated with UCB-MNCs compared to the baseline (-0.87; 95%CI: -1.2, -0.54) and control group (β: -0.58; 95%CI: -1.18, -0.11; Cohen's d: 0.36). The mean PEDI scores and mean CP-QoL scores in the two domains were higher in the experimental group compared to the control. The imaging data indicated that mean FA increased and MD decreased in participants of the UCB-MNC group indicating improvements in white matter structure. Lower back pain, headaches, and irritability were the most common adverse events within 24 h of treatment that were related to lumbar puncture. No side effects were observed during follow-up.
Conclusion: According to the results of this study, UCB-MNCs can be effective in improving the motor function of cerebral palsy and have the necessary safety.
Keywords: Cerebral Palsy, Mononuclear Cells, Umbilical Cord Blood

Ps-57:Injectable Microgel-Hydrogel Composite for Synergistic Therapy of Rectal Cancer Model Based on Sequential Delivery of Chemotherapy Agents

Pakian S[1*], Sadeghi Abandansari H[2], Nabid MR[1]

1. Department of Polymer and Materials Chemistry, Faculty of Chemistry and Petroleum Sciences, Shahid Beheshti University, Tehran, Iran
2. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: s_pakian@sbu.ac.ir

Objective: In situ formed injectable hydrogels based on Schiff's base reaction with pH-responsiveness and self-healing ability have great potential for anti-cancer drug delivery. The combination of multiple chemotherapeutics offers an effective approach to reduce disadvantages of single drug therapy, such as high dose and easy generation of multi drug resistance (MDR). We report herein the development of drug delivery system based on a novel Injectable Microgel-Hydrogel Composite for sequentially local co-delivery of Curcumin (Cur) and 5-Fluorouracil (5-FU). The prepared composite could be a promising drug delivery system with sustained-release action for up to 25 days for 5-FU and enhanced drug bioavailability. Furthermore, the activity of drugs released from composite matrix was evaluated by employing a human adenocarcinoma cell line. The combination therapy of antimetabolite and cytotoxic drugs using this delivery system offers a promising approach for improved cancer therapeutic efficacy. The synergistic inhibitory effects on the cell cycle progression and cell proliferation in rectal cancer cells were also confirmed. Taken together, this study demonstrates a promising dual-drug enhanced microgel-embedded injectable hydrogel for local dual drug delivery with sequential release behaviors by simple injection and thus provides a novel strategy for combination therapy of rectal cancer.
Materials and Method: Pluronic F127, gelatin, 5-Fluorouracil, Curcumin, EDC, NIPAm, acrylic acid, ammonium persulfate, N, N′-methylenebisacrylamide and MTT were purchased from Sigma-Aldrich. The combination of 5-FU loaded microgel and Gel-ADH solution was mixed with the pluronic micelle solution to obtain composite.
Results: 5-FU release could be sustained for more than a month from these composite hydrogels, significantly longer than that achievable using the microgels alone. The synergistic inhibitory effects were observed on the cell cycle progression and proliferation rate of in rectal cells using the dual drug loaded system.
Conclusion: In conclusion, this composite system is a synergism therapeutic delivery system that can achieve prolonged, sustained-release action for combination of chemotherapy agent, 5-FU and a selective COX-2 inhibitor, curcumin, against the human colon cancer cell line.
Keywords: Injectable Hydrogel- Microgel- Sequential Therapy- Colorectal Cancer- Localized Drug Delivery

Ps-58: Bone Marrow CD34 Positive Cells May Be Suitable for Collection after Death

Rafat A[1*], Dizaji Asl Kh[1], Mazloumi Z[2], Nozad Charoudeh H[3]

1. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: alirafat1370@gmail.com

Objective: Hematopoietic stem cells (HSCs) which are characterized with CD34+ phenotype, have a pivotal role in blood cell regeneration. They are located in lowest hypoxic areas in the bone marrow niches. This microenvironment protects them from DNA damage and excessive proliferation, whereas the oxygenated area driving cells out of quiescent state into proliferation. Given the resistance of HSCs to hypoxia, it is reasonable to imagine that they can survive for some time in the absence of oxygen.
Materials and Methods: Here, we evaluated CD34, Bax, Bcl-2, Bcl-xl, and p53 genes expression after death. Moreover, we established the ex-vivo development of HSCs using SCF, FLT3, IL-2, and IL-15 cytokines in culture system.
Results: Our finding indicated that although the most of the dead person's mononuclear cells were alive and adequately expressed the CD34 on their surfaces at the first day of isolation, the viability and CD34+/Ki-67 expression declined significantly after culture process.
Conclusion: Taken together, our finding indicated that the viability and CD34+ expression was acceptable on day 0 and could be used as a novel method for therapeutic purposes.
Keywords: CD34 Marker, Death Individual, Hematopoietic Stem Cells (HSCs), Oxygen Tension

Ps-59: Telomerase Inhibition Enhances NK Cells-Induced Apoptosis in Acute Myeloid Leukemia Cells

Rafat A[1*], Dizaji Asl Kh[1], Mazloumi Z[2], Nozad Charoudeh H[3]

1. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: alirafat1370@gmail.com

Objective: The need to develop new therapies in the treatment of malignancies has considered the utilize of immune cells especially natural killer (NK) cells as a suitable approach. Despite the valuable anti-tumor effects, using only NK cells has limited effects. In this study, we aimed to determine the effect of BIBR1532, an human Telomerase Reverse Transcriptase (hTERT) inhibitor, in promoting the cytotoxicity function of NK cells against acute myeloid leukemia (AML) cells.
Materials and Methods: AML cell line Kg-1a were cultured and treated with IC50 value of BIBR1532 for 48 h and subsequently with NK cells and its effects on cytotoxicity, cell proliferation and apoptosis were analyzed using AnnexinV /7AAD, Ki-67 assay, flow cytometry and real-time polymerase chain reaction (PCR) tests.
Results: Telomerase inhibition (TI) with IC50 value (57.64 μM) sensitizes AML cells and increased anti-proliferative effect of NK cells. Besides, TI augmented cytotoxic effect (IFN-γ and CD107-a) of NK cells against AML cells and significantly increased induction of apoptosis. Moreover, BIBR1532 and NK cells synergistically upregulated expression levels of Bax and Bad and Bax/Bcl-2 ratio and downregulated mRNA expression levels of Bcl-2, and Bcl-2/Bax ratio.
Conclusion: The combination of BIBR1532 and NK cells synergistically promoted its anti-proliferative effect and induction of apoptosis through targeting apoptosis related genes such as Bax and Bcl-2.

Keywords: Acute Myeloid Leukemia (AML), Apoptosis, Natural Killer Cell, Telomerase

Ps-60: Nitidine Chloride Suppresses The Cell Growth and Migration of Gastric Cancer Cells *In Vitro*

Rahbar M[1, 2*], Meyfour A[3], Moradi Sh[2]

1. Department of Developmental Biology, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Emails: sh.moradi@royan-rc.ac.ir, a.meyfour@sbmu.ac.ir

Objective: Gastric cancer (GC) is one of the most common types of cancer and the leading cause of cancer-related death worldwide. Nitidine chloride (NC), a natural benzo phenanthridine alkaloid compound, shows antitumor activity against various human cancers. However, the effect of NC on GC cell growth and migration has remained poorly investigated. Here, we aim to determine the biological effects of NC on the cell viability and migration of GC cells.
Materials and Methods: To examine the anti-cancer properties of NC using the human GC cell line AGS, several analyses were done, including MTS assay, Crystal Violet staining, and Live/Dead assay, together with wound healing assessment. The cell growth was investigated using MTS assay, crystal violet staining, and Live/Dead assay. Wound-healing assay was performed to investigate the migratory ability of GC cells.
Results: Based on the results of MTS viability assays, Live/Dead analysis, and Crystal Violet staining, we found that NC reproducibly inhibited cell viability of AGS GC cells compared to DMSO-treated control cells. Moreover, NC was able to significantly restrict the migration of the GC cells. Thus, NC is a potentially effective anti-cancer agent for GC treatment.
Conclusion: Our findings indicate that NC exerts strong inhibitory effects on human GC cells *in vitro*.
Keywords: Cancer Therapy, Cell Survival, Motility, Nitidine Chloride, Stomach Cancer

Ps-61: LGR5 As A Potential Therapeutic Target for Breast Cancer: A Systematic Review and Meta-Analysis

Razavi-Amoli SK[1*], Omrani-Nava V[3], Heydari K[1], Alizadeh-Navaei R[1], Kaidarova D[2]

1. Gastrointestinal Cancer Research Center, Non-communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran
2. Kazakh Research Institute of Oncology and Radiology, Kazakhstan, Kazakh Research Institute of Oncology and Radiology, Kazakhstan, Almaty, Kazakhstan
Email: reza_nava@yahoo.com

Objective: Since the emergence of precision medicine, the development of cancer stem cells (CSCs) has promoted the implementation of novel patient-centered therapies. Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), the so-called GPR49, plays a considerable role in the heterogeneity of tumors such as breast cancer. Breast cancer has emerged as the most common malignancy worldwide as of 2021. The implication of LGR5 in novel approaches to breast cancer therapy is a potential landscape for research. We aimed to assess the prevalence, prognostic, and therapeutic role of LGR5 in breast cancer.
Materials and Methods: We performed this systematic review and meta-analysis study using databases including Web of Science, Scopus, and PubMed. We searched these databases using LGR5 and Breast Cancer and related keywords based on the MeSH database until October 12, 2021. All studies that reported the prevalence of LGR5 overexpression via Immunohistochemistry (IHC) in breast cancer patients were included in this review. We applied the StATA and random effect models for data analysis.
Results: Finally, seven studies of 2632 breast cancer samples were reviewed in this study. The pooled prevalence of LGR5 high expression in breast cancer was 36 % (95%CI: 26-47.5%, I2= 95.5) and in triple-negative breast cancer was 48.6% (95%CI: 38.4-58.7%, I2= 0.0). There was a statistical significance in the correlation between LGR5 overexpression and ER+ breast cancer patients (pooled OR: 0.54, 95%CI: 0.32-0.92, I2: 62%). Furthermore, we observed that LGR5 overexpression is correlated with TNM staging of tumors (pooled OR: 5.06, 95%CI: 2.63-9.74).
Conclusion: In principle, the prevalence of LGR5 high expression in breast cancer particularly triple-negative types was considerable. Hence, we suggest that LGR5 can be a potential therapeutic target for breast cancer. The effectiveness of LGR5 in the tailored treatment of breast cancer needs further investigation.
Keywords: Breast Cancer, Cancer Stem Cell, Leucine-Rich Repeat-Containing G-Protein-Coupled Receptor 5 (LGR5), Tailored Medicine

Ps-62: Alkaline Phosphatase Localization in Spermatogonial Stem Cells and Embryonic Stem Cells and Its Association With Lin28 and Sall4

Reza E[*], Azizi H

Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
Email: h.azizi@ausmt.ac.ir

Objective: Alkaline phosphatases (AP) are a group of enzymes that hydrolyze phosphate-containing molecules under alkaline conditions. AP activity is an accepted marker for pluripotent stem cells. It has been shown that maintaining AP activity in the formation and maintenance of cells with cloning potential and self-renewal of undifferentiated embryonic stem (ES) cells are positively correlated. Lin28 and Sall4 proteins are expressed in testicular gonocytes as a transcription factor. They are expressed in germ cells and have different roles in maintaining the proliferation and differentiation of ES cells.
Materials and Methods: In the following paper, we examined the AP expression in Spermatogonial stem cells and ES cells with Alkaline phosphatase assay, and the expression of Lin28 and Sall4 with real-time and Fluidigm polymerase chain reaction (PCR), and PPI network analysis.
Results: This experiment and investigation showed that AP is positively expressed in spermatogonial stem cells and ES cells. Its expression is reduced and negatively expressed in differenti-

ated cells. Also, Lin28 and Sall4 have a positive and significant expression (P value <0.05) in these two cell populations and negative expression in differentiated cells. Also, these two genes, which were selected based on a direct effect on the regulation of AP expression, play a role in regulating spermatogenesis and each other's expression.
Conclusion: AP, Lin28, and Sall4 have a positive expression in spermatogonial stem cells and embryonic stem cells and can be used as markers for embryonic and germ cells. They are also involved in regulating spermatogenesis.
Keywords: Alkaline Phosphatase, Differentiation, Spermatogonial Stem Cells (SSCs), Transcription Factor

Ps-63: Senescence Human Endothelial Cells Produce Extracellular Vesicles with Distinct miRNAs Cargo

Rezaie J[1*], Abdolrahman Shaban Sh[2], Nejati V[2]

1. Solid Tumor Research Center, Cellular and Molecular Medicine Institute, Urmia University of Medical Sciences, Urmia, Iran
2. Department of Biology, Urmia University, Urmia, Iran
Email: rezaie.j@umsu.ac.ir

Objective: Extracellular vesicles (EVs) derived from senescence endothelial cells (ECs) contribute to endothelium dysfunction. We hypothesized whether EVs from human umbilical vein cells (HUVECs) contain distinct miRNAs cargo in a model of senescence *in vitro*.
Materials and Methods: Cellular senescent was induced by H2O2-treatment of HUVECs. The protein levels of P21, P53, and P16 were measured by western blotting analysis, while the expression of miRNAs -21 and miRNAs -126 were investigated by real-time PCR (q-PCR) in cells. Small EVs (sEVs) were isolated and characterized using AChE assay, western blotting, and transmission electron microscopy (TEM). The expression of miR-21 and miR-126 was measured by q-PCR. An *in vitro* wound healing assay was used to evaluate ECs migration rate upon incubation with sEVs.
Results: Data showed that the protein levels of P21, P53, and P16 were increased, however the expression of miRNAs -21 and miRNAs -126 was up-regulated and down-regulated in treated cells respectively (P value <0.05). Isolated sEVs were confirmed by western blotting and TEM. H_2O_2 increased sEVs production by treated cells (P value <0.05). A high level of miRNAs -21 concurred with a decrease in miRNAs -126 levels in sEVs of treated cells (P value <0.05). The wound healing rate of ECs incubated with sEVs of H_2O_2-induced cells was reduced (P value <0.05).
Conclusion: Our results showed that H_2O_2-induced HUVECs produced sEVs with distinct miRNAs cargo and function, proposing biomarker application in age-associated vascular disease.
Keywords: Cellular Senescence, Endothelial Cells, Extracellular Vesicles, H_2O_2

Ps-64: A comparative of Doxorubicin and Crab Derived Exosomes Effect in Breast Cancer 2- & 3-Dimensional Model *In Vivo*

Rezakhani L[*], Alizadeh M

Department of Tissue Engineering, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
Email: leila_rezakhani@yahoo.com

Objective: Cancer three-dimensional (3D) modeling, with tissue engineering assistance, will mimic an appropriate body system by modifying the tumor microenvironment. Exosome therapy has recently been used in cancer studies due to its anticancer effects. In this study, the effects of crab exosomes in a breast cancer (BC) 2D and 3D model were investigated *in vivo*.
Materials and Methods: In this study, female mice aged 6 to 8 weeks were used. By the Chitosan/β-glycerol phosphate hydrogel (Ch/β-GP) and 1×10^6 4T1 cells, a cancer 2D and 3D model were created in the mice. In both models, 1 mg/ml crab exosome and 5 mg/kg doxorubicin (DOX) were injected by intratumoral (IT), intravenous (IV), and intraperitoneal (IP) methods into mice's right flank in 3 intervals. After 21 days, the mice were sacrificed and the tissues were removed. Tumor weight and size were measured. VEGF, Bcl2, and P53 gene expression levels were assessed by real-time polymerase chain reaction (PCR). Nitric oxide (NO) secretion from the cancer 3D model was evaluated by Griess assay.
Results: Tumor size and weight in treated groups with exosome and DOX were reduced significantly (P value ≤0.001, P value ≤0.002, P value ≤0.02). Changes in VEGF, Bcl2, and P53 gene expression levels were less in the 3D model than in the 2D model. Decreased NO secretion was observed in all groups compared to the control group.
Conclusion: BC 3D model was shown greater drug resistance; this can be one of the features of a malignant tumor. Therefore, it can be used in cancer studies with greater confidence. Crab exosome was effective in BC inhibition.
Keywords: Breast Cancer, Crab, Exosome, Two-Dimensional, Three-Dimensional

Ps-65: *In Vitro* Evaluation of A Wound Dressing Composed of Human Foreskin Fibroblast on A Bovine Collagen Sheet

Roozbeh K[1, 2*], Saber M[2], Falah N[2], Hassani SN[2]

1. Department of Developmental Biology, Faculty of 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: sn.hassani@royan-rc.ac.ir

Objective: The skin is known as the largest organ of the body and also a barrier that protects the human against environmental changes. Burns is one of the most common injuries that occur to the skin. In this study, we used a commercial collagen sheet wound dressing material as a substrate for seeding of human foreskin fibroblasts (hFF) to create a biological wound dressing.
Materials and Methods: The hFF cells were provided by the Royan Stem Cell Bank and cultured in an appropriate medium. The bovine collagen sheet (Suprasorb® C) was cut out in dimensions of 0.2×.02 cm^2 and inserted into the wells of a 24-well plate. The cells were loaded at the density of 10^5 cells/well on the collagen sheet. The MTS assay was performed to analyze the viability and proliferation of the cells on the collagen sheet after one, two, and three days of incubation.
Results: The MTS assay showed significantly higher cell viability and metabolic activity in cell-laden collagen constructs in comparison to the control group (cell only) after one day in culture. There were no significant differences between groups after two- and three- days of incubation.
Conclusion: Based on the results of this study, it can be con-

cluded that the collagen sheet with hFF cells could be considered as a biological wound dressing. However, *in vivo* study on an animal model of burn can provide more information about the effectiveness of this biological bandage.
Keywords: Collagen Sheet, Human Dermal Fibroblast, Wound Dressing

Ps-66: Dual-Crosslinked Alginate Hydrogels with Shear-Thinning Property for 3D Bio-Printing Applications

Sadeghi Abandansari H[1*], Satarian L[2], Ghaempanah H[3]

1. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, 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 Material Science, Amirkabir University of Technology, Tehran, Iran
Email: l.satarian@royan-rc.ac.ir

Objective: Calcium-alginate hydrogel is often used for different biomedical applications, but its use as bio-ink in three-dimensional (3D) printing applications is somewhat limited due to its low mechanical properties. In this study, we synthesize a shear-thinning alginate hydrogel through dynamic imine chemistry and then use ionic crosslinking for post-printing stabilization using $CaCl_2$ solution.
Materials and Methods: Poly(ethylene glycol), sodium alginate, ethylene glycol, ammonium chloride, calcium chloride, and sodium hydroxide Alginate were oxidized to form alginate dialdehyde (ADA) and then mixed with 4-arm PEG-NH2 and pure alginate to form Schiff-base hydrogels. The post-printing reinforcement through the addition of $CaCl_2$ solution and second ionic crosslinking resulted in the printable hydrogel with stability and shape fidelity.
Results: Due to shear-thinning property and doul crosslinking, the hydrogels could be extruded for 3D printing of structures with high shape fidelity and stability to relaxation. Doul crosslinked hydrogels were degraded much more slowly than the single Schiff's base hydrogels. Additionally, the hydrogels showed very low cytotoxicity and hence can be used as a scaffold for cell encapsulation.
Conclusion: The stable tubes and grid structures were printed using these procedures with structural stiffness and shape fidelity. Double crosslinked hydrogels were stable for up to a month of incubation. The hydrogel also showed very low cytotoxicity and hence can be suitable for cell encapsulation. The double covalent-ionic crosslinked hydrogel could facilitate the printing of alginate hydrogel and address the challenges of mechanical stability upon printing.
Keywords: Schiff's Base, Shear-Thinning Hydrogel, Three-Dimensional (3D) Printing

Ps-67: Production and Purification of Human Recombinant GM-CSF in E. coli

Saleki P[1, 2*], Lachinani L[2], Dormiani K[2]

1. Department of Biology, Faculty of Science and Technology, ACECR, Institute of Higher Education, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, Isfahan, Iran
Email: k.dormiani@royan-rc.ac.ir

Objective: Granulocyte-macrophage colony-stimulating factor (GM-CSF) or CSF2 is an important hematopoietic growth factor and immune modulator. GM-CSF is produced in response to immune or inflammatory stimuli by activated cells of the hematopoietic system such as T cells, B cells, macrophages, and mast cells. Human GM-CSF (hGM-CSF) has 127 residues derived from a precursor containing a signal peptide. In the present study, the co-expression of thioredoxin fused with hGM-CSF was accomplished to improve the production of the soluble and active form of hGM-CSF in SHuffle T7 and BL21(DE3) E. coli strains as the host cell with enhanced capacity to correctly fold the protein.
Materials and Methods: The coding sequence of hGM-CSF was amplified by polymerase chain reaction (PCR) and finally subcloned into the pET-Duet1 vector. Thioredoxin (trxA) and His-tag sequences were added at the N-terminal of the hGM-CSF by designing the specific primers. After confirming the accuracy of the expression vector, the plasmid was transformed into SHuffle and BL21(DE3) E. coli. Transformed bacteria were cultured in LB broth containing ampicillin. Expression of the soluble hGM-CSF protein in LB medium was achieved by IPTG induction when the OD600 was approximately 0.5. At last, the recombinant protein was purified with Ni-affinity agarose and analyzed by the SDS-PAGE method.
Results: The accuracy of constructed vector (pET.Duet/trxA/His-tag/hGM-CSF) was confirmed and after its transformation, the optimal condition for the production of soluble recombinant hGM-CSF was obtained using 1 mM IPTG at 30 °C for 4 hours. Eventually, the recombinant protein was successfully isolated with Ni-affinity resin. The efficient purification of hGM-CSF with Ni-affinity agarose was confirmed by 15% SDS-PAGE in which a protein band with the size of 14.47 kDa was observed.
Conclusion: In the present study, efficient Shuffle E. coli expressing soluble recombinant hGM-CSF were obtained. The results showed that the recombinant protein efficiently produced the soluble hGM-CSF. In comparison to previous studies co-expression of TrxA reduced the induction time in Shuffle E. coli, resulting in the production of more soluble recombinant protein.
Keywords: Eshersi Coli (E. coli), Human Granulocyte-Macrophage Colony-Stimulating Factor (hGM-CSF), Thioredoxin (TrxA)

Ps-68: Regioselective Sulfated Chitosan Could Produce A Biocompatible and Antibacterial Wound Dressing with Low Inflammatory Response

Samani F[1*], Kazemi Ashtiani M[2], Karimi H[2], Shiravandi A[2], Baharvand H[3], Daemi H[2]

1. Department of Applied Cell Sciences, Faculty of Basic Sciences and Advanced Medical Technologies, Royan Institute, ACECR, 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: h.daemi@royaninstitute.org

Objective: The 6-O chitosan sulfate (CS) with naked amine

groups was synthesized via regioselective modification of chitosan (C) during which both crosslinking capacity and antibacterial properties of the C were remained intact. This was achieved by sulfation of the C under controlled acidic conditions using a chlorosulfonic acid/sulfuric acid mixture. Subsequently, a chemically crosslinked hydrogel of the CS was used as a wound dressing substrate.
Materials and Methods: The modified sulfate groups retained the biocompatibility of C and showed antibacterial effects against gram-positive and gram-negative bacteria. In addition, the presence of sulfate groups in the CS chemical structure improved its anticoagulant activity compared to the unmodified C.
Results: Both *in vitro* and *in vivo* enzyme-linked immunosorbent assay (ELISA) measurements showed that CS had a higher potential to bind and scavenger anti-inflammatory cytokines, including IL-6 and transforming growth factor-β (TGF-β), both of which play critical roles in the early stage of the wound healing process. After treatment of full-thickness wounds with CS hydrogels, the macrophage cells (c.a. 6×10^4 cells) expressed significantly more M2 phenotype markers compared to the C group (4.2×10^4 cells).
Conclusion: Furthermore, the CS hydrogel induced better re-epithelialization and vascularization of full-thickness wounds in mice compared to the C hydrogel during 30 days.
Keywords: Chitosan Sulfate, Immune Modulation, Regioselectivity, Wound Healing

Ps-69: Deciphering The Role of Progenitor and Stem Cells in Exocrine Pancreas Regeneration in Zebrafish

Shahbazi N*, Meyer Dirk

Institute for Molecular Biology, University of Innsbruck, Innsbruck, Austria
Email: Dirk.Meyer@uibk.ac.at

Objective: Exocrine pancreas displays an outstanding capacity for regeneration and cell fate plasticity. Using zebrafish models for exocrine cell ablation we previously discovered a novel rare cell population displaying features of immature exocrine pancreas cells and identified these cells as a source for tissue regeneration after virtually complete removal of mature acinar cells.
Materials and Methods: To better understand this progenitor pool, we now established novel transgenic tools in zebrafish for conditional ablating and for fluorescence-based lineage tracing. Further, FACS (Fluorescence-activated cell sorting) approaches to enrich progenitor cells were established as a requirement for single-cell RNA seq studies, aiming for a detailed molecular characterization of these cells under quiescence and regenerative conditions.
Results: Quantification of sectioned and dissociated healthy double (ptf1a: GFP/ela3l: E2Crimson) labeled pancreas suggests that the proportion of Ptf1a+/Ela3l- progenitor cells reduced from 5% in larva to <0.2% in the adult exocrine pancreas. To enable efficient FACS-sorting of these rare cells we established ela3l:mScarlet ablation line with bright and stable fluorescence which does not form aggregates and is easier to detect. Moreover, to trace Ptf1a+ cells during normal pancreas development and exocrine regeneration, we developed a Cre/lox-based reporter system for genetic cell labeling (ptf1a: CreERT2; hsp70: Cytbow). Finally, we established a line for conditional ablation of Ptf1a+-cells in order to determine if these cells are the only source for exocrine regeneration.
Conclusion: After confirming the existence and conservancy of Ptf1a+ cells even in the adult stage of zebrafish life, we now have all the necessary tools ready to study the molecular identity and role of these progenitor cells in the development and regeneration of the exocrine pancreas of zebrafish.
Keywords: Adult Stem Cells, Exocrine Pancreas, Regeneration

Ps-70: SMAD6 and IL4R Had A Prominent Role in Hydrogel Biomaterials Chemotherapy Drug Delivery in Glioma

Shomali H*, Rahmatipour H
Department of Medical Engineering, Central Tehran Branch, Islamic Azad University, Tehran, Iran
Email: Hanishli96@gmail.com

Objective: As is well known, gliomas are the deadliest of all brain tumors. Because of the blood-brain barrier (BBB) and the fact that tumor cells are different, giving systemic chemotherapy to treat glioma after surgery is ineffective and may even hurt the immune system. For this reason, the use of drug transporters such as hydrogels plays an important role in the passage of chemotherapy drugs through the blood-brain barrier. Evaluating the effect of hydrogels themselves on molecular mechanisms also plays an important role in the selection and type of chemotherapy drugs. In this study, the focus is more on the role of hydrogels in neurons and gliomas.
Materials and Methods: The molecular mechanisms of these hydrogel biomaterials and neurons were examined using a bioinformatics approach. There are numerous tools and databases used in this study to look at genes and protein products as well as molecular mechanisms as well as gene ontologies.
Results: 765 upregulated and 632 downregulated genes were observed in our data. In response to stress, NGF, FGF1, ERK/MAPK signaling pathways were demonstrated in these genes. Also, MAMDC2, PGF, FAM46A, NPC2, SMAD6, and IL4R were a prominent role in the effects of biomaterials hydrogels in neural cells.
Conclusion: Finally, in this investigation of the effects of biomaterials hydrogels in neural cells, the MAMDC2, IL4R, and SMAD6 genes had a more robust and better association with the effects of hydrogel carriers' chemotherapy drugs in glioma.
Keywords: Bioinformatics, Chemotherapy, Glioma, Hydrogel, Neural Cells

Ps-71: Comparison of POU5F1 Expression with Three Different Antibodies in Spermatogonia Stem Cell Populations of Neonatal and Adult Mice

Sojoudi K*, Azizi H

Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
Email: h.azizi@ausmt.ac.ir

Objective: Spermatogenesis is a complex and elaborate differentiation process and is vital for male fertility. Currently, many genes have been identified that affect the proper functioning of differentiation during spermatogenesis. POU5F1 (POU class 5 homeobox 1) is a crucial transcription factor for pluripotency and self-renewal, thus playing a vital role in accurate spermatogenesis.
Materials and Methods: In this experimental study, we examine POU5F1 protein localization by three various kinds of antibodies in the neonatal and adult mice testis section and isolated spermatogonial stem cells (SSCs) by immunohistochemistry

(IHC) and immunocytochemistry (ICC). Also, we examine the mRNA expression of POU5F1 in neonatal and adult mice SSCs by Fluidigm real-time polymerase chain reaction. The POU5F1 protein-protein network was constructed and gene enrichment analysis was performed.
Results: IHC and ICC data by all three antibodies showed that POU5F1 expression was much higher in neonate mice than in adult mice. Fluidigm analysis represented significantly higher expression (P value < 0.05) of POU5F1 in the adult testis in comparison to the neonate and a significant expression (P value < 0.05) level of germ cells gene POU5F1 in neonate SSCs (1-2 week) than 16–24-week SSCs. In silico data also show a strong association of POU5F1 with other markers involved in spermatogenesis.
Conclusion: These results indicate that POU5F1 is a necessary transcription factor of testicular germ cells that can be supportive for the analysis of germ cells development both *in vitro* and *in vivo*.
Keywords: Immunocytochemistry, Immunohistochemistry, POU5F1, Spermatogenesis, Spermatogonial Stem Cells (SSCs)

Ps-72: Itaconic Acid; as A Novel Immunometabolite, Differentially Regulates The Transcription of Autophagy Associated Genes in Rat Adipose Mesenchymal Stem Cells

Soroush F[1*], Tabandeh MR[2], Dayer D[3]

1. Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran
2. Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Stem Cells and Transgenic Technology Research Center (StTRC), Shahid Chamran University, Ahvaz, Iran
3. Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Cellular and Molecular Research Center (CMRC), Ahvaz, Iran
Email: m.tabandeh@scu.ac.ir

Objective: Itaconate is a novel regulatory immunometabolite that is synthesized by inflammatory macrophages and acts as an anti-inflammatory mediator by regulation of several metabolic and signaling pathways, in particular, the Nrf2 pathway. The immunometabolites can affect the stemness potency, differentiation ability, and viability of stem cells, but little is known about the critical function of itaconate on stem cell fate. In the present study, the effect of itaconate on viability and transcription of autophagy-related genes in rat adipose-derived mesenchymal stem cells (ADMSCs) was investigated.
Materials and Methods: In this experimental study, the ADMSCs were incubated with 125 μM and 250 μM dimethyl itaconate (DMI) for 24 hours or 48 hours. The expression of genes associated with autophagy (Atg12, Atg5, Beclin, LC3B, and P62) pathways was determined using quantitative polymerase chain reaction (qPCR) assay. The cellular level of phospho-Nrf2 protein was measured using the ELISA method.
Results: The results indicated that DMI increased the expression of Nrf2 protein and changed the expression of some autophagy-related genes (LC3B, Becline, and P62) in ADMSCs.
Conclusion: Because autophagy activation can protect stem cells under environmental stress, it is possible that itaconate, by converse regulation of the autophagy pathway, can affect the functions and viability of ADMSCs.
Keywords: Autophagy, Itaconic Acid, Rat Adipose Mesenchymal Stem Cells

Ps-73: Designing of Chitosan Collagen Composite Hydrogels Enriched with Human Platelet Lysates for Tissue Engineering

Taghinejad Z[1*], Shamsasenjan K[1], Nourdanesh Z[2], Abbasgholizadeh F[3], Shahparast S[4]

1. Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
3. Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Analytical Chemistry, University of Tabriz, Tabriz, Iran
Email: zahrataghinejad2020@gmail.com

Objective: Mesenchymal stem cells (MSCs) are multipotent progenitors and promising cell resources for regenerative medicine. MSCs can differentiate into a variety of cells, including osteoblasts, chondrocytes, adipocytes, fibroblasts, and cardiomyocytes. As most organs require the support of the extracellular matrix for their formation from cells, direct injection of the MSC into the damaged tissue is not capable of tissue repair and is not bioavailable. Thus, researchers are constantly on the lookout for a bioavailable environment that, *in vitro*, provides the optimal conditions for proliferation and differentiation of MSC to use in damaged tissue.
Materials and Methods: In this study, the composite hydrogel with the physiological pH and collagen/chitosan ratio of 5/1 was enriched with human platelet lysates and then encapsulated with adult human bone marrow (BM) derived stem cells (SCs).
Results: This collagen/chitosan hydrogel has been shown to provide MSCs with favorable growth environment. It also maintained cellular processes, including initial adhesion, mitosis, and differentiation.
Conclusion: Chitosan–collagen composite hydrogel materials can be used for MSC encapsulation and delivery, as well as in situ gel formation for tissue engineering.
Keywords: Chitosan, Collagen, Hydrogel, Mesenchymal Stem Cells, Regenerative Medicine

Ps-74: Fabrication and Evaluation of Conductive Polyaniline-Based Nanofibrous Scaffold for Skin Tissue Engineering

Tajedin R[*], Daraeinejad Z, Shabani I

Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran
Email: shabani@aut.ac.ir

Objective: One of the newest approaches to skin tissue regeneration is designing a scaffold with similar properties to the natural skin extracellular matrix. Conductive nanofibers can act as multifunctional scaffolds and their electrical nature can stimulate the proliferation of stem cells and increase the improvement of the wound healing process. In this study, a polyaniline (PANI) nanofibrous scaffold with a novel dopant was fabricated for enhancing skin tissue regeneration.
Materials and Methods: Nanofibrous scaffolds were fabricated through electrospinning. PLLA and PANI: dopant (1:1) were dissolved in chloroform and dimethylformamide, respectively. Then the solutions were mixed. Brilliant blue (BB) was used as an antibacterial dopant. Electrospinning was carried out with the high voltage power supply set at 30 kV, an air gap distance

of 30 cm, and a flow rate of 0.002 ml/h. The nanofibers were characterized using scanning electron microscopy (SEM), attenuated total reflection-Fourier-transform infrared spectroscopy (ATR-FTIR), and four-point-probe analysis. BB releasing was investigated by UV-visible spectroscopy.
Results: The presence of doped PANI in the scaffold was confirmed by ATR-FTIR. The morphology of PLLA/PANI-BB nanofibers was homogenous and beadless and the conductivity of this scaffold was acceptable. The BB showed an initial burst release, but after two days it had a sustain-release profile.
Conclusion: The prepared scaffold is suitable for skin tissue engineering due to its nanofibrous morphology and electrical conductivity. Furthermore, antibacterial property of the scaffold makes it a good option for wound healing.
Keywords: Conductive Nanofibers, Dopant, Electrospinning, Polyaniline, Skin Tissue Engineering

Ps-75: Investigating The Presence of Extracellular Mitochondria in The Content of The Stem Cells from Human Exfoliated Deciduous Teeth (SHED) in Normal Culture Conditions

Taraj F[1, 2*], Karbalaie Kh[2], Nasr-Esfahani MH[2]

1. Department of Biology, Faculty of Science and Technology, ACECR, Institute of Higher Education, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mh.nasr-esfahani@royaninstitute.org

Objective: Mitochondria are dynamic organelles that can enter the extracellular space under certain conditions as signaling organelles. Mitochondrial release from stressed cells has been debated for years, while the presence of mitochondria in the extracellular space of cells under normal culture conditions is unknown. Stem cells from human exfoliated deciduous teeth (SHED) and their secretome, due to their ease of access and wide therapeutic applications, have been regarded as a specific candidate for cell therapies. Therefore, the present study aimed to investigate the presence of extracellular vesicles containing mitochondria or naked mitochondria in a culture medium of SHED in normal culture conditions.
Materials and Methods: SHED with a density of 45 $\times 10^4$ cell/cm^2 were cultured in DMEM supplemented with 10% FBS. After the cells reached 90% confluency, conditioned media were cleared from cellular debris by centrifuging at 400 g for 10 minutes. Mitochondria isolation was performed throughout 10000 g, 10 minutes, at 4 °C. The pellet was solved in PBS (free calcium and magnesium) and prepared for transmission electron microscopy (TEM) and Dynamic Light Scattering (DLS) analysis.
Results: We demonstrate that cells could release mitochondria under normal culture conditions. The presence, morphology, and size of mitochondrial-related particles were detected with TEM and DLS.
Conclusion: Based on the TEM and DLS results, in normal culture conditions, mitochondria are released from the cells in the form of naked, vesicular, and even swollen mitochondria.
Keywords: Extracellular Vesicles, Mesenchymal Stem Cells, Mitochondria

Ps-76: A PEI-Alg Polyelectrolyte Micelle Containing Melittin to Break Down Multidrug Resistance in Breast Cancer Cell Line

Tavakolnejad Z[1, 2*], Mirahmadi-Zare SZ[2], Dormiani K[2]

1. Department of Biology, Faculty of Science and Technology, ACECR, Institute of Higher Education, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mirahmadi_zare@royaninstitute.org

Objective: Melittin peptide (MLT), the main constituent of bee venom, has a great ability to digest the extracellular matrix (ECM) and stimulate the cells of the immune system. Therefore, if it can be successfully loaded into smart nanoparticles that preserve it before delivery to cancer cells, it is expected to be able to overcome multidrug resistance (MDR) by digesting ECM proteins and then inducing cell death. In particular, various mechanisms for MLT cytotoxicity have been reported in different types of cancer cells, including cell cycle changes, effects on proliferation and/or growth inhibition, and induction of apoptosis and necrosis by activation of caspases and ECM metalloproteinases. In addition, the co-delivery of melittin with odder chemotherapeutic drugs can synergistically increase their efficiency in the treatment of the cancer cell line. Therefore, a polyelectrolyte core-shell micelle based on polyethylene imine-alginate (PEI-Alg) was designed with the ability to load melittin along with a secondary cargo (GFP vector as a reporter and drug model). Results confirmed the micelle stability for safe transport of MLT and its smart delivery onto the multidrug resistance breast cancer cell line (MDA-MB-231). In addition, ECM protein expression was significantly different in the treated group compared to the control.
Materials and Methods: In this study, a shell-core polyelectrolytic nanosystem was designed by the microemulsion method. Polyethyleneimine and GFP vector were used as nuclei and alginate was used as a shell to carry the peptide. Nanosystems were investigated for size, load, stability, loading efficiency, and drug release. The efficacy of melittin-containing nanosystems in the expression of ECM factors and induction of cell death on the MDA-MB-231 cancer cell line is then evaluated.
Results: The findings showed that encapsulating the drug in the micellar nanocarrier, increased the stability of the nanosystem in the circulatory system and decreased its release before reaching the surface of the cancer cell with an acidic environment. In addition, due to the ability of melittin to digest ECM and increase the permeability of the tumor and its placement in the nanosystem shell, it increased the drug delivery to cancer cells and increased drug efficiency.
Conclusion: Our study successfully declares that melittin-loaded in polyelectrolyte nanocarrier with micelle coating had more anti-cancer effects than free melittin. The present studies showed that polyelectrolytic nanosystems are suitable carriers for melittin, compare to the free form.
Keywords: Co-Delivery Drug to Cancer, Extracellular Matrix, Micelles, Melittin, Polyelectrolyte Nanocarrier

Ps-77: Process Development in Isolation and Culture of Human Monocytes from Cord Blood; Simple and Cost-Effective Method

Torabi Sh[1, 2*], Nouri M[3], Zarabi M[2, 3], Vosough M[2]

1. Department of Applied Cell Sciences, Faculty of Basic Sciences and Advanced Medical Technologies, Royan Institute, ACECR, Tehran, Iran
2. Department of Regenerative Medicine, Cell Science Research

Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Research and Development, Royan Stem Cell Technology Company, Cord Blood Bank, Tehran, Iran
Email: masvos@royaninstitute.org

Objective: Macrophages are multifunctional immune cells that are widely used for immunological research. To study the function of macrophages *in vitro*, umbilical cord blood is a convenient and non-invasive source that provides sufficient numbers of monocytes and macrophages. Monocyte isolation procedures vary widely in terms of purity, yield, cost, and technical difficulty. We aimed to find an optimized protocol for monocyte isolation from cord blood and compare the effect of three different variables in monocyte differentiation and culture techniques: 1. Cell culture media; 2. Serum sources and 3. Cell culture systems on cellular phenotype, adhesion, and viability.
Materials and Methods: We used double density gradient centrifugation techniques (Ficoll® and hyperosmotic Percoll®) for monocyte isolation and flow cytometry analysis of light scattering and/or expression of pan surface markers, such as CD3, CD14, and CD19 to determine cell viability and purity. After purification, cells were cultured in adhesion preventing tissue culture plates in either RPMI-1640 or DMEM-F12 or IMDM culture medium supplemented with either 0.5-10% fetal bovine serum (FBS), human serum (HS), or human platelet lysate (hPL).
Results: We introduce a novel, reproducible, and inexpensive isolation method that yields monocytes with high purity (from70 to 98%). Also, the results showed that monocyte adhesion to the plate is significantly increased in the presence of a low concentration of serum, and IMDM containing 5% hPL can be the best option for macrophage cultivation and differentiation.
Conclusion: This optimized method is a simple and cost-effective scale-up method to provide uniform and pure monocytes for studies of the innate defense system.
Keywords: Cell Viability, Innate Immune System, Monocyte, Umbilical Cord Blood

Ps-78: The Small Molecule Enoxacin Prohibits The *In Vivo* Growth and Tumorigenicity of Esophageal Cancer Cells

Torkian H[1, 2*], Yazdani movahed A[1, 2], Moradi Sh[2]

1. Department of Developmental Biology, Faculty of 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: sh.moradi@royan-rc.ac.ir

Objective: Esophageal carcinoma (EC) is one of the most lethal cancers worldwide. Several cancer types, including EC, show global downregulation of microRNAs, i.e., non-coding RNAs involved in post-transcriptional gene regulation, to enable higher invasiveness. Therefore, we aimed to analyze if enoxacin, a microRNA-enhancing small molecule, could inhibit EC growth *in vitro* and *in vivo*.
Materials and Methods: To investigate enoxacin (124 μM) in EC cells (KYSE-30 line) *in vitro*, MTT viability assay, Crystal Violet staining, and Live/Dead assay were performed 72 hours post-treatment. The migratory ability of the cells was carried out using a wound healing assay. A sub-cutaneous mouse model of EC was established using KYSE-30 and immunocompromised mice. The cells (4×10^6) were injected into nude mice 72 hours after treatment with enoxacin (1 mg/kg). Tumor tissues were removed and fixed in 10% buffered formalin. Next, paraffin-embedded specimens were cut into serial sections and stained with hematoxylin and eosin. Data were analyzed using GraphPad Prism and ImageJ (P value <0.05).
Results: We found that enoxacin reduced the growth and viability of EC cells *in vitro*. We also observed that enoxacin was able to restrict the migration of the cells. Furthermore, the tumor formation ability of EC cells in mouse models was decreased in enoxacin-treated cells compared to the control groups (n = 5). Particularly, no tumors (n = 4) or only small tumors (n = 1) were observed in treated mice, judging from histological evaluations. Finally, the tumors in the treated groups were less aggressive than the control group.
Conclusion: Enoxacin inhibits the growth and migration of EC cells *in vitro* and in animal models of EC.
Keywords: Carcinogenesis, Esophagus, Esophageal Squamous Cell Carcinoma, miRNA Pathway, RNAi

Ps-79: Optimizing Electroporation Conditions to Improve The Transfection Efficiency of Stem Cells from Apical Papilla

Yazdani R[1, 2*], Karamali F[2], Eghbalsaied Sh[2, 3], Nasr Esfahani MH[2]

1. Department of Biology, Faculty of Science and Technology, ACECR, Institute of Higher Education, Isfahan, Iran
2. Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
3. Department of Animal Science, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
Email: frkaramali@royaninstitute.org

Objective: Stem cells from apical papilla (SCAP) are among the most widely used stem cells derived from the biowaste source. They have been recently considered as a therapeutic candidate with immunomodulatory potential as well as secretion of a wide variety of neurotrophic factors. But some challenges of genetic manipulation such as the low transfection efficiency of SCAP are one of the problems that need to be solved. Electroporation which is defined as the process of pore formation in cell membranes via the application of an electric field is a relatively safe and simple method to introduce vectors into the cells.
Materials and Methods: During this study, the cells were maintained in a normal conditioned medium. In order to electroporation, a single cell suspension was prepared and an aPX461 vector containing EGFP reporter with different voltages 100, 150, 200, 250, and 300V, double pulse, and pulse width of 10ms were introduced to the SCAPs. 24 hours later, the rate of cell death and expression of the reporter gene was analyzed with a flow cytometer and fluorescence microscopy.
Results: Our results showed that 250V provides the highest efficiency of SCAPs around 10%.
Conclusion: It provided a valuable set-up to improve the transfection efficiency of mesenchymal cells, especially SCAP.
Keywords: Electroporation, Stem Cells from Apical Papilla, Transfection Efficiency

Ps-80: The Fluoroquinolones Enoxacin, Moxifloxacin, and

Gemifloxacin Differently Influence The Growth of Human Pluripotent and Non-Pluripotent Cells

Yazdani Movahed A[1, 2*], Torkian H[1, 2], Moradi Sh[2]

1. Department of Developmental Biology, Faculty of 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: sh.moradi@royan-rc.ac.ir

Objective: Human embryonic stem cells (ESCs) have a great potential for cell therapy. However, they might give rise to tumors even if they are differentiated before transplantation. It has been reported that augmenting the RNAi pathway resists tumorigenesis. Here, we aim to compare the potential of the RNAi enhancer enoxacin and two non-RNAi enhancing fluoroquinolones, i.e., gemifloxacin and moxifloxacin, in inhibiting the growth of undifferentiated ESCs, to provide potentially safer ESC-based cell therapies.

Materials and Methods: The human ESC line Royan H6 was maintained in DMEM/F12, 20% KOSR, 1% non-essential amino acids, 1% ITS, 1% L-Gln, and bFGF (12.5 ng/ml). Human dermal fibroblasts (HDFs) were cultured in high-Glc DMEM and 15% FBS. Phase-contrast imaging, Live/Dead staining, and MTT viability assays were used to analyze the effect of enoxacin (124 μM), gemifloxacin, and moxifloxacin (0~124 μM) on the cells.

Results: Based on our phase-contrast imaging, enoxacin reduced the growth of ESCs but not of HDFs. Additionally, in contrast to enoxacin, gemifloxacin and moxifloxacin considerably inhibited the growth of both ESCs and HDFs in different concentrations. Besides, MTT assays revealed that enoxacin had an ESC-selective inhibitory effect whereas genifloxacin and moxifloxacin inhibited the viability of both cell types. These results were confirmed using Live/Dead analyses.

Conclusion: The RNAi enhancer enoxacin appears to be a selective inhibitor of human ESCs. In contrast, gemifloxain and moxifloxacin induced cell death in both ESCs and HDFs.

Keywords: Apoptosis, Fluoroquinolone, Stem Cell Therapy, Teratoma, Pluripotency

Ps-81: The RNAi Enhancers Ciprofloxacin and Ofloxacin Selectively Inhibit the Growth of Human Pluripotent Stem Cells: An Efficient Approach Towards Safe Cell Therapy

Yazdani Movahed A[1, 2*], Torkian H[1, 2], Moradi Sh[2]

1. Department of Developmental Biology, Faculty of 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: sh.moradi@royan-rc.ac.ir

Objective: Human embryonic stem cells (ESCs) hold great promise for regenerative medicine. However, there is a potential tumorigenicity associated with the residual undifferentiated ESCs. The enhancement of the RNAi pathway has been found to be detrimental to tumorigenesis. Here, we aim to exploit the potential of two RNAi enhancers, ciprofloxacin and ofloxacin, to inhibit the growth of undifferentiated ESCs, thereby ensuring the safety of ESC-based cell therapy.

Materials and Methods: The human ESC line Royan H6 was cultured in DMEM/F12, 20% KOSR, 1% ITS, 1% non-essential amino acids, and 1% L-Gln in the presence of bFGF (12.5 ng/ml). Human dermal fibroblasts (HDFs) were maintained in high-Glc DMEM and 15% FBS. To examine the effect of ciprofloxacin and ofloxacin (both, 124 μM) on the cells, we used phase-contrast imaging, MTT viability assays, and Live/Dead staining.

Results: The phase-contrast imaging revealed that both ciprofloxacin and ofloxacin decreased the growth of human ESCs. In contrast, the growth of HDFs remained intact in response to the treatments. In addition, our MTT assays indicated that both chemicals sharply reduced the viability of human ESCs as opposed to HDFs. Finally, we confirmed the diminished viability of human ESCs upon treatment with ciprofloxacin and ofloxacin using Live/Dead analysis. Notably, no inhibitory effects on the HDF viability were observed post-treatment, as evidenced by a large number of live (green) cells in both the treated and untreated groups.

Conclusion: The RNAi enhancers ciprofloxacin and ofloxacin exert a potent suppressive impact on the growth of human ESCs but not fibroblasts.

Keywords: Cell Death, Fluoroquinolone, Pluripotency, Teratoma, Stem Cell Therapy

Ps-82: Effect of Hydrogel Stiffness on Biological Features of Normal and Cancer Cells

Yousefi P[1], Mohajer H[1], Zare Gachi M[2], Daemi H[3], Mashayekhi M[2]

1. Farzanegan 3 High-School, Karaj, Iran

2. Department of Biomaterials, Zharfandishan Fanavar Zistbaspar (ZFZ) Chemical Company, Tehran, Iran

3. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Email: h.daemi@royan-rc.ac.ir

Objective: Cancer is a major challenge in the field of health care, which is spreading, especially in developing countries. Although different treatments have been developed to cure it, cancer cells are eventually able to resist against these treatments. During tumorigenesis, the interaction between cancer cells and the tumor micro-environment often leads to ECM stiffness. Therefore, the aim of this study is to investigate the effect of stiffness as an important mechanical parameter of ECM on the proliferation and migration of normal and cancer cells.

Materials and Methods: We used alginate as a natural hydrogel due to its desirable properties such as biocompatibility, ease of gelling, low biodegradability, negative electrical charge, and bio-inert nature to encapsulate the cells. Initially, calcium alginate beads were prepared with two different molecular weights of alginate, and their hydrolytic stability was studied in PBS. After selecting the medium molecular weight as the appropriate option, the calcium alginate beads with different stiffness contents of 0.7 - 43.5 kPa, were prepared through controlling the gelation time and concentration of the crosslinker.

Results: Finally, the effect of hydrogels' stiffness on proliferation and migration of both normal and cancer fibroblast cells was probed by the cell encapsulation process. Both MTT and scratch assay results showed that both normal and cancer cells showed a good responsivity to the stiffness of the hydrogels.

Conclusion: Our results well showed that both molecular weight and stiffness can affect the proliferation and migration of normal and cancer cells.
Keywords: Alginate, Cancer, Extracellular Matrix, Hydrogel, Stiffness

Ps-83: Expression Profiling of Prostate Cancer Stem Cells in Tumor Development: Possible Therapeutic Implications

Zaersabet M[1*], Rahimi Tesiye M[2]
1. Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
2. Faculty of Life Science and Biotechnology, Shahid Beheshti University, Tehran, Iran
Email: maryamrahimi563@yahoo.com

Objective: Prostate cancer stem cell (PCSC) is one of the subcategories of cancer cells with the ability of self-renewal, flexibility, onset, and maintaining of tumorigenesis. The purpose of this study is to investigate the gene expression patterns of prostate stem cells compared to normal tissue. Given the role of these cells in stability and tumor development, the results of this study can reveal the differentiation mechanism of prostate cancer stem cells to cancer cells, which leads to potential therapeutic strategies to target genes controlling this differentiation.
Materials and Methods: The data relevant to normal tissues and cancer stem cells were collected from GEO database with GSE55945 and GSE19713. For each category, 3 sets of data were used. The applied platform was [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. Data analysis was done by R software. After entering the commands related to the directory setting, showing microarray images, and drawing histogram, data were normalized. Subsequently, heatmap commands were inputted in order to observe the gene expression patterns.
Results: Our results showed that there is a discernible difference between the gene expression patterns of these two groups. To elucidate, a high proportion of PCSC genes experienced an upregulation and/or downregulation pattern.
Conclusion: Evaluation of PCSCs and their differentiation mechanism can contribute to valuable discoveries in prostate cancer treatment and heighten the knowledge about this specific area of science.
Keywords: Differentiation, Gene Expression, Prostate Cancer Stem Cell (PCSC), R Software, Tumorigenesis

Ps-84: The Evaluation of The Antitumoral Immune Response by Using Mouse Pluripotent Stem Cell in Breast Cancer Mice Model

Zarghami SM[1, 2*], Badri M[1, 2], Saber M[2], Shekari F[2], Ebrahimi M[2], Hassani N[2]

1. Department of Developmental Biology, Faculty of 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: sn.hassani@royan-rc.ac.ir

Objective: More than a century ago, it was illustrated that immunization with embryonic materials can lead to the rejection of a transplantable tumor. On the other hand, studies over the past few decades have shown that embryonic stem cells (ESCs) and their induced counterparts (induced pluripotent stem cells; iPSCs) have similar cellular and molecular properties to cancer cells and could stimulate the immune system, trigger an antitumor response and reject tumor cells after transplantation.
Materials and Methods: The mouse ESC line C17 and breast cancer cell line 4T1 were provided by Royan Stem Cell Bank and cultured in their appropriate medium. The C17 cells were prepared in two inactive and lysate forms before vaccination. For inactivation, the cells were irradiated with a 15 Gy gamma-ray. Cell lysates were obtained after five cycles of freeze-thawing. BALB/C mice were divided for immunization into four groups including C17 in lysate and inactive forms, adjuvant, and PBS. Immunization was done three times one week apart. Mice were challenged with 4T1 cells one week after the last immunization. Tumor growth was monitored every 3 days. Finally, 21 days after the tumor challenge, all mice were sacrificed. The histological assay was performed in all groups.
Results: The tumor size was significantly reduced in mice immunized with C17 cell lysate compared to the PBS group (p value <0.01). Also, the frequency of CD8+ T cells in the tumor tissues significantly increased in the lysate vaccinated group.
Conclusion: The results indicated that immunization with lysate of C17 ESCs could stimulate the immune response against a murine breast carcinoma model.
Keywords: Antitumor Responses, Breast Cancer, Cell Lysate, Mouse Embryonic Stem Cell

Ps-85: Systemic Toxicity Assessment of Two Skin Substitutes in Rat Models of Skin Wound Healing

Khalaj Asadi Z[1, 2*], Gholipour N[2], Hajizadeh Saffar E[3], Hassani N[2]

1. Department of Developmental Biology, Faculty of Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sn.hassani@royan-rc.ac.ir

Objective: Before each clinical trial of advanced therapy medicinal products (ATMPs), preclinical studies on animal models of diseases are required to characterize the mechanisms of action and evaluation the toxicity, the dosage, and the route of administration of the new medicine. The aim of this study was to investigate the systemic toxicity of two skin substitutes, RoyinSheet and RoyinGraf, in both subacute and subchronic phases of wounds in rat. RoyinSheet, a cryopreserved cultured epidermis (human keratinocyte sheet), and RoyinGraf, a bilayered living cellular construct from human fibroblast and keratinocyte were developed in the ATMP-TDC center at Royan Institute.
Materials and Methods: The potential systemic effects of two skin substitutes were studied after transplantation of RoyinSheet in burn wounds and RoyinGraf in full-thickness wounds in rats. In each subacute and subchronic toxicity study, the male and female Wistar rats were randomly assigned into three groups (treatment, sham, and control). The behavior, body weight, and food- and water intake were assessed during these phases. Two weeks (for subacute phase) and 13 weeks (for subchronic

phase) post-transplantation, the animals were sacrificed and urine and blood samples and also major organs (liver, kidneys, heart, lung, Bone marrow (BM)) were harvested from each rat. All tissues were processed and stained with hematoxylin and eosin (H&E) and BM was stained with Giemsa for light microscopic examination.
Results: During the study, death was observed in four animals within 24 hours post-surgery in the sham group, maybe due to the anesthesia. No animal death occurred in the treatment groups. Based on the two-way ANOVA statistical analysis, no significant differences were observed in parameters of body weight, water, and food intake in both subacute and subchronic phases. Urine analysis did not show pathologic effects in treatment versus sham and control groups. Hematological, biochemical, and blood coagulation tests indicated that the triglyceride parameter in the subacute phase of RoyinSheet product and platelet-, glucose- and urea parameters in the subacute phase of RoyinGraf product were significantly different between sham and treatment groups in comparison to healthy controls (one-way ANOVA statistical analysis, P value <0.05). However, there was not any significant difference between sham and treatment groups. Also, no pathologic abnormities were seen in the structure of vital organs (liver, lung, heart, and kidney) and BM in both subacute and subchronic phases. No signs of histological changes such as inflammation, abnormal tissues, or necrosis were observed in the heart, lung, kidney, liver, and BM in each group.
Conclusion: Overall, the results suggest that local transplantation of skin substitute RoyinSheet and RoyinGraf in both sexes of Wistar rats do not cause toxicity during the subacute and subchronic periods of time.
Keywords: Skin Substitutes, Systemic Toxicity, Wound Healing

Evaluation of the effect of Fluoropyrimidine on HNF4alpha protein by molecular docking method

Farshid Aaliazar

Cellular and Molecular Biology, Islamic Azad University Shahre Qods, Tehran, Iran

Objective: Fluoropyrimidine is one of the drugs used in chemotherapy for stomach cancer. Studies show that this drug can affect the HNF4alpha protein and reduce the progression of cancer. In this study, we try to investigate the effect of this drug binding to the protein HNF4alpha Investigate the interaction between this protein and the drug by molecular docking
Material and Methods: In this descriptive-analytical project, we first downloaded the most suitable three-dimensional structure of HNF4alpha protein in terms of resolution and number of suitable chains from the Uniprot site in pdb format. We see some specifications of this protein below. This protein has 5 chains A, C, G, E, I was. resolution =3.70A Then, protein chains were examined using Chimera software. The most suitable chain was c chain, which had more amino acids than other chains, and the largest protein chain was HNF4alpha. Through this software, water molecules and all We removed the solvents from this chain and hydrogen ions and charge bar were added to the chain and finally saved in pdb format HNF4alpha protein in the next step, we downloaded the structure of Fluoropyrimidine from Pubcheem site in SDF format. The information about Nitidine Chloride was as follows: Molecular formula: C4H3FN2 Molecular weight: 98.08 3D Conferm Fluoropyrimidine To perform the docking process, pyrx software was used. In this software, after entering the protein as a receptor and the drug Fluoropyrimidine as a ligand, we obtained the binding site through the Deepsite, the specifications of which were as follows: Center c= 25.00 Center y=25.00 Center z=25.00
Results: After docking with Pyrex software, 10 models were suggested, the first three models being the best docking modes, the results of which were obtained in the table below model Binding affinity kcal/mol Rmsd 1 --4.00 kcal/mol 0.00 2 -3.7 kcal/mol 2.713 3 -3.7 kcal/mol 16.769
Conclusion: According to Docking results, it can be concluded that Fluoropyrimidine with poor negative binding energy cannot bind well with HNF4alpha protein, but there is a proper orientation of the drug within the protein according to RMSD = 0 and this drug cannot be a good drug. To bind to the HNF4alpha protein
Keyword: Fluoropyrimidine, HNF4alpha, Molecular docking

Developing Trabecular and Cortical Bone Microstructure Thru Decellularized Extracellular Matrix Composite Hydrogels

Ebrahimi Sadrabadi A[1], Hosseini S[1, 2*], Baghaban Eslaminejad M[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 Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: eslami@royaninstitute.org

Objective: There have been many efforts to develop bone-like construct to mimic the trabecular and cortical bone microstructure precisely. Novel bone tissue engineering approaches have offered decellularized bone extracellular matrix (dECM) as a promising biomaterial that holds the biochemical and biophysical cues of native bone. The composite hydrogel contains dECM and other bone imitative biomaterials would provide the other cues. Therefore, we reasoned that calcium phosphate cement (CPC) and silk fibroin-derived peptide (SFp) could form the composite hydrogel with dECM to imitate the trabecular/cortical bone microstructure.
Materials and Methods: To this end, the solubilized dECM combined with 1.5 % (V/V) CPC and 150 µg/ml SFp have been prepared separately and seeded with adipose derived mesenchymal stem cells. Then, the morphological and geometrical analysis of CPC/dECM and SFp/dECM constructs were performed using scanning electron microscopy energy dispersive spectrometry (SEM-EDS). Also, the mechanical strength analysis of resulted constructs was performed.
Results: Interestingly, the MSCs have secreted the sufficient extracellular matrix thru both CPC/dECM and SFp/dECM. The SEM-EDS analysis manifested that CPC/dECM hydrogel imitated the trabecular-like structure and SFp/dECM hydrogel mimicked the cortical-like structure. Furthermore, the mechanical property analysis of CPC/dECM hydrogel compared to SFp/dECM manifest a higher compression strength.
Conclusion: Altogether, this study demonstrates that the bone-like construct which imitates the bone microstructure specifically can be designed in detail.

Keywords: Bone tissue engineering, silk peptide, dECM, CPC, bone microstructure

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