Abstracts of

Royan International Twin Congress

7th Congress on Stem Cell Biology & Technology

7-9 September 2011

Royan Institute

Cell Science Research Center

Tehran, Islamic Republic of Iran

Cell Journal(Yakhteh)

Guide for Authors

Aims and Scope: The "***Cell Journal*** *(Yakhteh)*" is a publication of Cellular Sciences Research Centre, the Royan Institute. It is published in English. The aim of the journal is to disseminate information through publishing the most recent scientific research studies on exclusively cellular, molecular and other related topics. ***Cell Journal*** *(Yakhteh)*, has been certified as a quarterly publication by Ministry of Culture and Islamic Guidance in 1999 and was accredited as a scientific and research journal by **Health and Biomedical Information (HBI)** Journal Accreditation Commission in 2000. **This journal is a member of the Committee on Publication Ethics (COPE).**
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* Example: Edelman CL, Mandle CL. Health promotion throughout the life span. 2nd ed.ST Louis: Mosby; 1998; 145-63.

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* Example: Eftekhari Yazdi P. Comparison of Fragment Removal and Co-Culture with Vero Cell Monolayers on Development of Human Fragmented Embryos. Presented for the Ph.D., Tehran. Tarbiyat Modarres University. 2004.

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* Example: Harnden P, Joffe JK, Jones WG, editors.Germ cell tumors V.Proceedings of the 5th Germ cell tumors conference ;2001 Sep 13-15; Leeds,UK.New York:Springer; 2002.

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IN THE NAME OF GOD

Gone But not Forgotten

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

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Mohamadreza Baghaban Eslaminejad

Dear Friends and Colleagues,

It is my great honor to inform you that the 7th congress on Stem Cell Biology and Technology will be held in Tehran this coming September 7-9, 2011. Please accept this invitation to join us, and many of your professional colleagues at this outstanding congress.

Currently, stem cells are considered a hot topic of investigation and offer many promising options for the future treatment of major diseases such as cancer, nervous system diseases, damaged organs and degenerative diseases. The aim of the 7th congress on Stem Cell Biology and Technology is to move stem cell research forward. The intent of this congress is to provide an innovative and comprehensive overview of the latest research developments in the fields of regenerative medicine and stem cells.

I appreciate all those who intend to participate, including scientists, organizations, universities and research centers. I would like to express my thanks to all individuals that have worked so hard to make the 7th congress on Stem Cell Biology and Technology a successful event.

Best regards,
Mohamadreza Baghaban Eslaminejad
Congress Chairman
Stem Cell Biology and Technology Congress

Animal Biotechnology

Is-1: A Reverse Transcriptase-Dependent Mechanism Plays Key Roles in Early Embryogenesis and Tumourigenesis

Spadafora C

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In mammalian cells, endogenous reverse transcriptase (RT) is encoded by two families of retrotransposons, i.e. LINE-1 (Long Interspersed Nuclear Elements) and endogenous retroviruses (ERVs), which account for about 17% and 10% of the human genome, respectively. Emerging evidence from our laboratory suggest that a LINE-1-encoded RT plays regulatory roles in both early embryonic development and in cell transformation.
Early pre-implantation mouse embryos are endowed with an RT activity. We have found that inhibition of this activity, either induced by RT-inhibitory drugs, or by microinjecting LINE-1-targeted antisense oligonucleotides into zygotes, causes early developmental arrest in preimplantation stages in association with subverted gene expression profiles. On these grounds, LINE-1-encoded RT activity emerges as a component strictly required for early murine development implicated in control of gene expression.
An RT-dependent mechanism is also implicated in modulation of cell proliferation and differentiation. Drug-mediated inhibition of RT activity, or RNA interference-mediated silencing of human active LINE-1 elements, reduce cell proliferation and promote differentiation in a variety of cancer cell lines. Concomitantly, RT inhibition causes modifications in chromatin architecture, associated with a global reprogramming of expression profiles. On discontinuation of RT inhibition, however, transformed cells resumed their original proliferation and de-differentiation features. Together, these data suggest that an RT-dependent epigenetic machinery acts as a global regulator of genome expression, both in physiological and in pathological developmental processes.

Is-2: Sperm-Mediated "Reverse" Gene Transfer

Spadafora C

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Mature spermatozoa of virtually all animal species can spontaneously take up foreign DNA molecules, internalize them in nuclei and further deliver them to embryos upon oocyte fertilization. By exploiting this feature, genetically transformed embryos and/or born animals of a variety of species have been generated with variable degrees of efficiency.
In an effort to study this process we have identified a sperm endogenous RT activity that can reverse-transcribe the internalized molecules in cDNA copies: exogenous RNA is reverse-transcribed in a one-step reaction, whereas DNA is first transcribed into RNA and subsequently reverse-transcribed. The newly synthesized cDNAs are delivered from spermatozoa to oocytes at fertilization and further propagated throughout embryogenesis and in tissues of adult animals. *In vitro* fertilization (IVF) assays using spermatozoa that were pre-incubated with RNA from a beta-galactosidase (β-gal) expression construct or with a plasmid harboring a green fluorescent protein (EGFP) retrotransposition cassette showed that the reverse-transcribed sequences are underrepresented (below 1 copy/genome), mosaic distributed in tissues of adult individuals, transmitted in a non-Mendelian fashion from founders to F1 progeny, transcriptionally competent, variably expressed in different tissues and temporally transient, as they progressively disappear in aged animals. Based on these features, therefore, the reverse-transcribed sequences behave as extrachromosomal, biologically active retrogenes capable of inducing novel phenotypic traits in animals. This RT-dependent mechanism likely originates from LINE-1 retroelements and generates transcriptionally competent retrogenes in sperm cells which are then transmitted to offsprings and propagated as newly acquired genetic traits in tissues of adult animals. These data strengthen the emerging view of a novel transgenerational genetics as the source of a continuous flow of novel epigenetic and phenotypic traits, independent from those associated to chromosomes. The distinctive features of this retrotransposon-based phenomenon is compatible with a Lamarckian-type adaptation. We have called this phenomenon: Sperm-Mediated "Reverse" Gene Transfer.

Stem Cells

Is-3: Ethics of Animal Research

Agoramoorthy G

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The meaning of "ethics" is hard to pin down, and the views many people have about ethics are questionable. As a matter of fact, ethics is the moral philosophy that involves systematizing, defending, and recommending concepts of right and wrong behavior. Ethics is simply not a philosophical exercise, and it evolves and emerges from the realities of daily life. Then the question, however is, would it be ethical to use animals

in biomedical experiments? Perhaps we all know that millions of animals that range from mice to monkeys are being used in various types of animal experiments. These "lab rats" are killed yearly either in biomedical laboratories or for various product testing. Animal rights activists have been pressuring governments around the world to enforce stricter restrictions on the use of animals in biomedical research. Whether animals should be used in experiments benefiting humanity has become a matter of public debate. The proponents argue that animal testing has a crucial role in medical advances and people have a better quality of life because of such advances leading to new medicines and treatments. But the hardcore opponents of animal testing argue that any experiment involving non-human animals are not only cruel but also unethical regardless of its benefit to humanity. Unfortunately, it is difficult to reconcile ideological differences between these two independent groups and to harness their combined efforts in alleviating animal suffering and improving animal welfare in research facilities. Nonetheless, the biomedical community has an option to work with the more moderate animal welfare groups to develop appropriate ethical and welfare guidelines to harness their combined efforts in alleviating animal suffering and improving animal welfare standards in laboratories. It is time to remember one thing: nothing in the ecosystem is redundant - each and every organism including humans has a role to play in the process of sustaining the balance of nature. Therefore it is argued that the lives of all organisms have value and are certainly worthy of recognition and respect. Unfortunately, we often forget this basic reality. The renowned Irish playwright George Bernard Shaw once said, "When a man wants to murder a tiger he calls it sport; when the tiger wants to murder him he calls it ferocity".

Is-4: Is It Ethical to Use Zoo Animals in Research?

Agoramoorthy G

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On behalf of the Ethics and Welfare Committee of the South East Asian Zoos Association, I have conducted zoo evaluations since 1998 to improve ethical and animal welfare standards in countries such as Thailand, Indonesia, Vietnam, Malaysia, Singapore, Philippines and Taiwan. The aim was to identify, rectify and prevent ethical and welfare related problems in zoos. The most controversial ethical issues facing zoos today are the acquisition of animals for breeding, disposal of surplus animals, animal care and husbandry, and use of animals in research. Although the use of animals in entertainment (animal shows and photography) is common in the region, only a few cases of animal experimentations were observed. One of the member zoos involved in the Assisted Reproductive Technologies by conducting experiments on therapeutic cloning with the collaboration of a local university. While visiting the zoo's quarantine building, about 30 wild-caught long-tail macaques were seen in small cages and their welfare did not meet the regional minimum standards. The floor area and height of ceiling and climbing structures fell short of the minimum requirements for monkeys. The low-ranking monkeys that were harassed by dominant individuals did not have a place to hide causing undue stress. Some macaques displayed abnormal behavior such as stereotypy and self-mutilation. A rapid ethics and welfare evaluation yielded a score of only two out of five basic internationally recognized freedoms of animal welfare. Can the zoo justify keeping some monkeys for research while the rest displayed to educate people about their conservation? As a matter of fact, the South East Asian Zoos Association does not recommend zoos to engage in invasive research using monkeys as models since non-human primates are high priority target for animal welfare issues globally.

Is-5: Alpha-Cell-Mediated Beta-Cell Regeneration in The Endocrine Pancreas

Collombat P

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Is-6: New Insight in The Alpha-to-Beta-Cell Transdifferentiation in The Endocrine Pancreas

Collombat P

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The identification and characterization of the genetic determinants underlying endocrine pancreas morphogenesis and regeneration may potentially aid the design of cell replacement therapies to treat type 1 and 2 diabetes mellitus. In this context, we previously found that, during the course of pancreas development, the transcription factors Pax4 and Arx play a crucial role in specifying endocrine precursor cells towards the β- and α-cell lineages, respectively. Indeed, in mice lacking Arx, the β- and δ-cell lineages are favoured at the expense of the α-cell fate, while the total number of endocrine cells remains unchanged. Conversely, mice lacking Pax4 display the opposite phenotype. Interestingly, we also observed that the forced expression of Arx in adult β-cells resulted in their conversion into cells presenting α- or PP-cell features. Based on these results, we investigated whether the opposite conversion was achievable and therefore generated mice conditionally expressing the Pax4 gene. Our data indicated that the ectopic expression of Pax4 in pancreatic α-cells

resulted in their conversion into cells displaying a β-cell phenotype. Importantly, the subsequent glucagon shortage was associated with a compensatory glucagon-positive cell neogenesis requiring the reactivation of the pro-endocrine transcription factor Ngn3. Markedly, such continuous cycle of neogenesis and re-differentiation of α-cells into β-like cells proved to be capable of restoring a functional β-cell mass and euglycemia in streptozotocin-treated animals.

Is-7: Micro and Nanomaterials to Manipulate Stem Cells

Ferreira L

Biomaterials and Stem Cell-Based Therapeutics Research Group at CNC, Center of Neurosciences and Cell Biology, University of Coimbra, Coimbra, Portugal
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The administration of some biomolecules to differentiate stem cells poses significant challenges because of their poor water solubility, short half-life, and potentially undesired side effects. One example is retinoic acid (RA), which interacts with members of the hormone receptor super-family, including the RA receptor (RAR), located in the cell nuclei. RA is rapidly metabolized by cells and has low solubility in aqueous solutions. In addition, the use of this biomolecule in an in vivo setting for the differentiation of resident stem cells remains elusive. We anticipate that nanoparticles (NPs) can be an excellent platform to ensure protection and intracellular transport of RA. Several RA NPs formulations have been described in the last years; however, no formulation was used for the intracellular delivery of RA in order to drive the differentiation of stem cells. Herein we describe a RA-containing NP formulation (RA+-NP) able to cross the cell membrane of subventricular zone (SVZ) stem/progenitor cells and release its payload intracellularly. This process drives the differentiation of stem cells into neuronal cells without affecting cell viability.We report a novel method to modulate the differentiation of SVZ cells into neurons involving the use of RA+-NPs.
NPs with an average diameter of 200 nm were prepared, having a significant high RA payload (86 ± 28 μg of RA per mg of NPs) and a positive net charge (+15 mV).
The NPs presented higher degradation rates at endossomal (pH = 5 - 6) than at physiological pH (pH = 7.4), likely due to the protonation of PEI amine groups (in excess relatively to the sulfate groups in DS) and the concomitant repulsion between positive charges. The RA+-NPs can be taken up rapidly by SVZ cells (first 5 hours) under the tested conditions and localize in the overall cell cytoplasm after 18 hours. RA+-NPs for concentrations below 100 μg/mL do not exert a cytotoxic effect as evaluated by TUNEL, propidium iodide uptake and active caspase 3 immunodetection. Incubation of SVZ cells with RA+-NPs at concentrations ranging between 0.1 and 1 μg/mL (corresponding to a RA payload of 1.2-12 ng/mL) induced an increase of NeuN+ cells (neurons). Importantly, RA+-NP-conditioned medium (CM) is unable to promote the differentiation of stem cells indicating that neurodifferentiation is only mediated by internalization of the RA+-NPs. In addition, RA+-NPs are pro-neurogenic for lower concentrations of RA as compared to RA dissolved in the differentiation medium, highlighting for the first time the importance of drug spatial positioning and concentration in terms of stem cell differentiation. Finally, we show that the internalization of RA+-NPs contribute for a high percentage of functional neuronal cells as shown by an increase of intracellular calcium following KCl but not histamine stimuli.

Is-8: Stem Cells and Biomaterials for The Treatment of Ischemic Diseases

Ferreira L

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It is estimated that 15% of the diabetic patients have non-healing foot ulcerations. Recent data show that healthy adult stem/progenitor cells improve the healing of diabetic chronic wounds. However, in autologous setting, the use of adult stem cells from diabetic patients raise some issues since the stem cells have impaired properties due to ageing and disease. Human umbilical cord blood (UCB) can be a potential source of healthy endothelial progenitor cells for the healing of chronic wounds in diabetic patients. In the present study, we investigated the use of UCB-derived CD34+ cells to promote the healing of diabetic wounds when administered topically in a fibrin gel. To enhance the therapeutic effect of CD34+ cells, they were co-cultured with CD34+- derived endothelial cells (ECs). Our results show that these ECs have higher angiogenic and prosurvival properties than typical differentiated ECs such as human umbilical vein endothelial cells (HUVECs).
Cell survival and differentiation is improved by co-culturing CD34+ cells with CD34+-derived ECs. Our results show that fibrin gels are permissive environments for CD34+ cells attachment. Quantitative LIVE/DEAD and MTT analyses show a significant increase in cell viability and proliferation for CD34+ cells co-cultured with CD34+-derived ECs ($n=6$, $p<0.01$ or $p<0.001$). In contrast, HUVECs did not affect the viability of CD34+ cells ($n=6$, $p>0.05$), indicating that the pro-survival effect is specifically mediated by CD34+-derived ECs. Importantly, addition of CD34+-derived EC-conditioned medium to cell constructs formed by CD34+ cells resulted in a significant increase in cell viability ($n=6$, $p<0.001$). Our results suggest that the pro-survival effect of CD34+-derived ECs on CD34+ cells

is mediated, at least in part, by bioactive factors released from the ECs, which activated ERK signalling pathway. We have identified IL-10 and IL-17 as important mediators in this process. Our results also show that CD34+ cell differentiation into ECs is significantly improved in the presence of CD34+-derived ECs. Co-transplantation of CD34+ cells with CD34+-derived ECs improved wound healing. Our results indicate that diabetic chronic wounds treated with a combination of both types of cells encapsulated in a fibrin gel have improved healing compared to wounds treated with gel containing only stem cells. We demonstrated that the co-culture system secreted factors, which increased neovascularization and decreased inflammatory reaction.

Is-9: Direct Reprogramming of Fibroblasts into Cardiomyocytes by Defined Factors

Ieda M

Department of Clinical and Molecular Cardiovascular Research, Keio University School of Medicine, Tokyo, Japan
Email: ieda@cpnet.med.keio.ac.jp

Loss of terminally differentiated cardiomyocytes due to myocardial damage is irreversible and current therapeutic regimes are limited. The reprogramming of fibroblasts to induced pluripotent stem (iPS) cells and further successful differentiation of iPS cells into cardiomyocytes provide a revolutionary paradigm in heart regeneration. However, generating sufficient iPS-derived cardiomyocytes that are pure and that can be delivered safely and timely remains challenging. We recently found that a combination of three developmental transcription factors (Gata4, Mef2c and Tbx5) rapidly and efficiently reprogrammed post-natal cardiac or tail-tip fibroblasts into differentiated cardiomyocyte-like cells. Induced cardiomyocytes expressed cardiac-specific markers, had a global gene expression profile similar to cardiomyocytes, and contracted spontaneously. Fibroblasts transplanted into mouse hearts after transduction of the three factors also differentiated into cardiomyocyte-like cells. Thus, direct reprogramming of endogenous cardiac fibroblasts into cardiomyocytes might be a new method for regenerative therapy.

Is-10: Heart Regeneration Using Stem Cells and Direct Reprogramming Technology

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Heart disease is the leading cause of morbidity and mortality in developed countries. Cardiomyocytes are terminally differentiated cells and their regenerative capacity is very limited in the adult heart. Cell replacement therapy is an attractive option to repair injured hearts. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are pluripotent and capable of indefinite expansion *in vitro*, suggesting they are ideal cell types for cell replacement therapy. Although ES cells might be ethically and immunologically problematic for clinical use, iPS cells may overcome such issues. During the past several years, significant advances in iPS cell generation technology, cardiac differentiation, and cell purification protocols were achieved for the development of stem cell-based heart therapies. Moreover, the discovery of iPS cells has sparked a new idea which is direct conversion of mature cell types into another cell type. I will overview the recent research achievements and discuss future challenges in stem cell-based cardiac generation and direct cardiac reprogramming technology for heart regeneration.

Is-11: Defining and Predicting Pluripotent Behavior in Human ES and IPS Cells

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Human pluripotent stem cells (hPSCs) represent a powerful and potentially unlimited source for disease modeling and for applications in regenerative medicine. While multiple protocols have been developed to derive specific cell types *in vitro*, there is considerable variability in the efficiency of generating differentiated lineages among independent hESC and hiPSC lines. Currently there is no method available that predicts a priori the differentiation properties of a given cell line. Therefore extensive optimization of individual lines is required to obtain comparable results across lines. Such variability is particularly problematic for the use of patient-specific iPSC lines in disease modeling as it can mask disease-related phenotypes.
Here, to identify markers that predict neural differentiation potential, we characterized 33 human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) lines that were maintained simultaneously. Quantitative analysis of miR-371-3 expression prospectively identified hESC and hiPSC lines with low versus high neurogenic propensity and in vivo dopamine neuron engraftment potential. Predictive markers corresponded to genes previously shown to distinguish mouse ESCs from epiblast-stem cells (EPI-SCs) and, surprisingly, showed more variability in expression among hESC versus hiPSC lines. Neural induction in lines with low neurogenic propensity was more dependent on exposure to BMP inhibitors. Transient over-expression of KLF4 was sufficient to alter neurogenic behavior and markers of pluripotent state. Suppression of miR-371-3 in KLF4-transduced cells rescued neural differentiation propensity demonstrating both predictive and functional roles for miR-371-3

in human pluripotent stem cell behavior.
Many of the differentially expressed markers represent genes previously known to discriminate mouse ESCs versus EPI-SCs, and we further demonstrate that such markers can be used to predict *in vitro* differentiation behavior of a given cell line, which suggest the existence of different level of pluripotent status among hESC and hiPSC lines. Furthermore, we demonstrate that transient over-expression of KLF4 is capable of altering differentiation behavior and pluripotent marker expression, which possible imply the conversion of pluripotent status by simple genetic manipulation. Our findings indicate that the variable differentiation propensity among hESC and hiPSC lines is in part due to differences in pluripotent state and offer a simple strategy to predict and manipulate cell line properties.

Is-12: Discovery of Potential Therapeutic Compouns for Familial Dysautonomia Using Patient-Specific and Symptom-Relevant iPSC Derived Neural Crest Precursors

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Patient-specific human induced pluripotent stem cells (hiPSCs) offer unprecedented promise in disease modeling and drug discovery by providing unlimited number of symptom-relevant cells. Taking advantage of our novel methodology of direct differentiation and prospective isolation of neural crest (NC) from human pluripotent stem cells, we successfully modeled Familial Dysautonomia (FD) with hiPSC technology. The results from these studies offered previously unknown clues for disease pathogenesis and allowed us to validate several candidate drugs. To extend our approach beyond the validation of a few candidate compounds, we present here our data on the first high-throughput screening (HTS) drug discovery effort performed in FD-iPSC derived symptom-relevant NC precursors.
FD-iPSCs differentiated into NC using our standard MS-5 differentiation protocol and purified by flow cytometry using HNK1 (CD57) antibody. Enriched NC population was further expanded for 2 weeks yielding > 0.5 x 109 cells that were could be cryo-preserved prior to performing HTS assay. To establish culture condition in 384-well-plates, we optimized seeding density, proliferation rate and coating reagents. Furthermore, by using commercially available kits, automated protocols for RNA extraction and qRT-PCR were developed to measure level of wild type (WT) and mutant (MU) IKBKAP expression. We selected qRT-PCR as the primary HTS readout given that reduction in transcriptional levels of wild-type IKBKAP is closely correlated with FD disease symptoms.
Using our optimized culture conditions in 384-well-plates and automated qRT-PCR protocols, we tested the impact of chemical compound libraries (>7000 compounds, FDA-approved drugs and natural products) on WT and MU IKBKAP expression in patient-specific and symptom-relevant NCs. Expression levels were normalized to housekeeping gene and all assays were performed in three biological repeats per compound. We identified a total of 43 primary hits that were subjected to various validation studies, cytotoxicity assays and dose-response studies (12 concentrations). Finally, 8 candidate compounds were selected based on increased level of WT IKBKAP transcript, which was significantly correlated with enhanced IKAP protein. In further functional analysis, we found that more than 3 compounds showed the rescue of mis-regulation of autonomic gene expression (ASCL1 and SCG10). However, we could not find any significant increase in the migration defect in FD-NC.
Our drug discovery effort using symptom-relevant and patient-specific cells is a proof of concept demonstration for HTS with disease-specific hiPSC lines. We hope that our proposed paradigm (specification/isolation of symptom-relevant cell type, disease modeling and HTS for drug discovery) will prove suitable for other iPSC based disease models and lead to novel treatments for many currently intractable diseases. In conclusion, our studies illustrate the potential of using patient-specific hiPSCs for HTS-based drug discovery.

Is-13: Induction of Functional Hepatocyte-Like Cells from Mouse Fibroblasts by Defined Factors

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Is-14: Bioengineered Niches to Instruct Stem Cell Fate

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Proper tissue maintenance and regeneration relies on intricate spatial and temporal control of biochemical and biophysical microenvironmental cues, instructing stem cells to acquire particular fates, for example remaining quiescent or undergoing self-renewal divisions. Despite rapid progress in the identification of relevant niche proteins and signaling pathways using powerful in vivo models, to date, many adult stem cell populations cannot be efficiently cultured *in vitro* without rapidly differentiating. To address this challenge, we and others have been developing biomaterial-based approaches to display and deliver stem cell regulatory signals in a precise and near-physiological fashion, serving as powerful artificial microenvironments to study and manipulate stem cell fate both in culture

and in vivo. In this talk I will highlight recent efforts in my laboratory to develop microarrayed artificial niches based on a combination of biomolecular hydrogel and microfabrication/robotic technologies. These platforms allow key biochemical characteristics of stem cell niches to be mimicked and the physiological complexity deconstructed into a smaller, experimentally amenable number of distinct signaling interactions. The systematic deconstruction of a stem cell niche may serve as a broadly applicable paradigm for defining and reconstructing artificial niches to accelerate the transition of stem cell biology to the clinic.

Is-15: Probing Single Hematopoietic Stem Cell (HSC) Fate Decision-Making in Artificial Niches

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The remarkable ability of stem cells to renew themselves and to give rise to specialized cell types has raised huge hope for their clinical application. However, our limited understanding of the mechanisms that regulate stem cells poses a substantial hurdle for their clinical use: Since we know relatively little about how stem cells function, it is not surprising that we also struggle in growing them in a cell culture dish; most likely an important requirement for stem cell-based therapies.
I believe that one cause of this problem is the inadequacy of tools that we have available to study stem cells at the single cell level. That is to say, even after careful prospective isolation based on immunophenotype, individual stem cells of a well-defined population behave highly heterogeneous in culture: some cells divide rapidly and others more slowly; some self-renew while others begin to differentiate; some can give rise to more lineages than others, etc. Because of this inherent variability, population-based studies of stem cells are essentially 'black boxes' and often unable to accurately address key biological questions, such as defining the discrete development steps from a single stem cell to a complex population of specialized cells (lineage development), or elucidating the mechanisms that regulate symmetric versus asymmetric divisions of stem cells.
In this talk I will highlight recent efforts in my lab to address this important problem by developing and applying microfluidic technology to sequentially capture single HSC after multiple divisions to assess their fate, and in particular the symmetry of division, by multigene single cell qRT-PCR.

Is-16: Adipose Tissue-Derived Progenitors for The Engineering of Osteogenic and Vasculogenic Grafts

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In the context of a cell-based approach to bone tissue engineering, one of the open challenges lies in the generation of constructs in clinically relevant sizes which not only have osteogenic properties upon implantation, but also can be rapidly vascularized, in order to allow cell survival even in the inner regions and consequent successful engraftment. Among the different strategies to improve vascularization of engineered grafts, the co-delivery of endothelial cells has been proposed to generate a vascular network which would then anastomose with the host vasculature. Towards this ambitious goal, the following topics will be presented and critically discussed.

A. Utilization of human adipose tissue-derived stromal vascular fraction (SVF) cells

SVF cells from human excised fat or lipoaspirates contain a relatively large fraction of mesenchymal progenitors (colony forming unit-fibroblasts represent about 5% of the total nucleated cells, corresponding to about 100-fold higher amounts/ml than in human bone marrow aspirates), as well as endothelial lineage cells. We have recently reported that direct perfusion of freshly harvested SVF cells through porous ceramic scaffolds (8 mm diameter, 4 mm thick cylinders) for 5 days generated constructs which, upon ectopic implantation in nude mice, generated bone tissue and capillaries of human origin functionally connected to the host vessels.

B. Vascularization of scaled-up implants

We then tested whether the extension of the approach to larger, clinically sized constructs (up to 1 cm diameter, 1 cm thick cylinders) could support graft vascularization and thereby allow better survival of the implanted cells in the construct core. Upscaled tissues engineered as described in (A) and implanted subcutaneously in nude rats displayed a rather uniform deposition of bone tissue, and included blood vessels of human origin. The efficiency of graft vascularization and cell survival critically depended on the presence of endothelial lineage cells.

C. One-step, intraoperative engineering of osteogenic and vasculogenic grafts

In order to facilitate transfer and use of the strategy to the clinic, we addressed whether constructs with similar properties could be generated within times compatible with an intraoperative use, namely bypassing the *in vitro* culture phase for 5 days. Human SVF cells were thus loaded into ceramic scaffolds using a fibrin gel (within 2-3 hours from the harvest time) and immediately subcutaneously implanted in nude mice. Due to the inert ectopic environment and the lack of an appropriate *in vitro* 'priming', the cells were not capable to form bone tissue, although they maintained the vasculogenic properties. Experiments are ongoing to investigate whether an orthotopic environment or the co-delivery of a small dose of BMP-2 (250 ng/graft) could induce the implanted tissues to become osteogenic.

We demonstrated using different models that human SVF cells from adipose tissue have intrinsic osteogenic/vasculogenic capacity. The attractive possibility to generate the grafts within a time frame compatible with a surgical operation warrants further validation in preclinical and clinical tests

Keywords: Tissue Engineering, Mesenchymal Stem Cells, Vascularization

Is-17: Three-Dimensional Expansion of Human Bone Marrow-Derived Mesenchymal Stromal Cells

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Bone marrow-derived mesenchymal stem cells, or marrow stromal cells (MSC), are receiving an increasing experimental and clinical interest, owing to the large degree of plasticity, to the capacity to modulate the immune system or the phenotype of cancer cells, as well as to the potential use for treatment of genetic or immunologic pathologies and in the emerging field of regenerative medicine. For any of these purposes, given the low frequency among bone marrow nucleated cells (around 0.01%), MSC are typically expanded by sequential passages in monolayer (2D) cultures, which is associated with a progressive and dramatic reduction of their 'early progenitor' functions. In this study, we propose a novel paradigm for the efficient expansion of human MSC within porous, three-dimensional (3D) scaffolds under direct alternate perfusion, which totally bypasses the 2D culture step. The relevance of the developed method will be discussed in the following different fields

A. Generation of osteogenic grafts

Human MSC cultured directly under perfusion for up to 3 weeks can generate highly and reproducibly osteogenic grafts, as assessed by ectopic implantation in nude mice. The possibility to include features of automation and control into the perfusion bioreactor system opens the prospective of a streamlined, standardized and possibly cost-effective manufacture of MSC-based osteogenic grafts.

B. Expansion for cellular therapy

Human MSC expanded directly under perfusion for up to 3 weeks, enzymatically extracted from the scaffold pores, reproducibly maintain higher clonogenic properties and more efficient multilineage differentiation capacity as compared to cells grown in 2D for the same time or the same number of doublings. Microarray analysis of 2D- and 3D-expanded MSC strains validated the consistency of the process across several donors and highlighted several candidate genes which may be involved in the maintenance of early progenitor properties.

C. Engineering of stromal microenvironments

The 3D perfusion system offers the opportunity to generate multicell 3D co-culture models, which may be used to study interactions of MSC with different cell types (e.g., hematopoietic, endothelial, tumorigenic) under physiologically relevant conditions.

Direct culture of bone marrow-derived cells within 3D perfusion systems is relevant not only to improve efficacy, automate and streamline the process of MSC expansion for a variety of experimental or clinical applications, but also to maintain MSC in a controlled 3D environment, as a model to establish stem cell niches and study physiological interactions with other cell types. The method has recently been shown to be also applicable to MSC derived from other sources (e.g., adipose tissue).

Keywords: Tissue Engineering, Bioreactor, Mesenchymal Stem Cells

Is-18: Generation of Functional Organs from iPS Cells: Toward the Next Generation of Regenerative Medicine

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Stem cell based regenerative medicine has been a focus of attention worldwide. In particular, recent development of the iPS cell technology has enabled generation of patient's pluripotent stem cells (PSCs), opening up the way to regenerative medicine using patient's own PSC-derived cells. However, current stem cell therapy mainly targets diseases that can be treated by cell transplantation, such as Parkinson's disease or diabetes mellitus. The complexity of organogenesis hinders *in vitro* generation of organs derived from a patient's PSCs, an ultimate goal of regenerative medicine. To address this issue, we hypothesized first that a niche for organogenesis is vacant in post-blastocyst mutant mouse embryos genetically precluded from development of a certain organ, and second that injected induced pluripotent stem cell (iPSC)-derived cells would colonize this niche, compensate for the developmental defect, and form a donor-induced organ in vivo. We tested this hypothesis using Pdx1-/- blastocysts (pancreatogenesis-disabled) and blastocyst complementation technique. When wild type rat iPSCs were injected into mouse Pdx1-/- blastocysts, defective cells were totally replaced and pancreas was formed almost entirely by injected rat iPSC-derived cells. Chimeric mice of Pdx1-/- genotype survived to adulthood without any sign of diabetes. Generation of organs using rat iPSCs and blastocyst complementation in vivo provides a new strategy for understanding organogenesis and a novel approach for organ supply.

Is-19: Heterogeneity and Hierarchy within The

Most Primitive Hematopoietic Stem Cell Compartment

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Objective: HSCs supply all blood cells throughout life by making use of their self-renewal and multi-lineage differentiation capabilities. However, the mechanism of self-renewal and many other biological properties of HSCs largely remain unknown. In order to address these issues, we established a protocol to prospectively isolate HSCs from mouse bone marrow (BM) with the use of monoclonal antibodies and 4-color FACS cell sorting (Osawa et al. Science 1996). Against our expectation from human studies, mouse HSC were exclusively enriched in CD34-negative (CD34-) rather than CD34-positive (CD34+) fraction among c-Kit+Sca-1+Lineage Marker- (KSL) cells. CD34-KSL cells are very rare cells representing 1 out of 25000 bone marrow mononuclear cells. Although small in number, these cells have enormous proliferative and multi-lineage differentiation capacity enabling long-term bone marrow reconstitution of lethally irradiated mice by transplantation of single CD34-KSL cells. Use of these highly enriched HSC has enabled analysis of individual HSC both in vivo and *in vitro*, contributing to better understanding of the mechanisms of aging, self-renewal, and differentiation of HSC. Indeed, analyses of individual purified HSCs or limiting doses of whole bone marrow transplanted into irradiated animals have revealed marked functional heterogeneity in HSCs with regard to repopulating activity, self-renewal activity, and *in vitro* colony-forming activity. Different patterns in lineage reconstitution by individual HSCs have also been observed. Of interest is that the donor-derived myeloid/lymphoid ratio in reconstituted mice reportedly indicates the degree of self-renewal potential in transplanted HSCs. If a variety of HSCs exist, HSCs may exhibit and define hierarchical organization within the most primitive hematopoietic compartment. Alternatively, heterogeneity of HSCs may be generated during development of the hematopoietic system and remain fixed thereafter. To further address questions of functional diversity and hierarchy in HSCs, HSC subsets with distinct properties must first be isolated prospectively. To identify candidate cell-surface markers that could prospectively identify functionally distinct HSCs, we screened a large number of antibodies and identified those with heterogeneous staining patterns on CD34-KSL cells. These candidates were then tested for functional differences in vivo. In this way, we found that expression of CD150 could be used to enrich for long-term repopulating cells (LTRCs) with distinct reconstitution kinetics patterns. CD34-KSL cells were subdivided into CD150high, CD150med, and CD150-neg fractions, and the functions of these cells were compared at the clonal level using single-cell transplantation and cultures.

Materials and Methods: C57BL/6 mice congenic for the Ly5 locus (B6-Ly5.1 mice) were bred and maintained at Sankyo Labo Service Co (Tsukuba, Japan). B6-Ly5.2 mice were purchased from Japan SLC (Hamamatsu, Japan). All procedures were approved by the Institutional Animal Care and Use Committee. Screening of antibodies. Bone marrow cells were obtained from 8- to 10-week-old B6-Ly5.1 mice and were suspended in phosphate buffered saline (PBS). Low-density cells (< 1.077 g/ml) were isolated by density gradient centrifugation, and were stained with each additional antibody for screening. For staining of fluorescein isothiocyanate (FITC)- or Alexa488-conjugated additional antibodies, CD34-KSL cells were identified by phycoerythrin (PE)-conjugated anti-Sca-1(D7, eBiosicence), PE-Cyanin7 (PE-Cy7)-conjugated anti-c-Kit (2B8, eBiosicence), Alexa647-conjugated anti-CD34 (RAM-34, eBiosicence), and an allophycocyanin-Cy7 (APC-Cy7)-conjugated antibodies or a biotinylated anti-lineage marker antibody cocktail (anti-CD4, -CD8, -CD11b, -CD45R, -CD127, -Gr-1, and -TER119 antibodies, eBioscience). The biotinylated anti-lineage marker cocktail was developed using streptavidin-conjugated APC-Cy7. For staining of PE-conjugated antibodies, as when PE-conjugated anti-CD38 (90) or PE-conjugated anti-CD150 (TC15-12F12.2: BioLegend) was used, CD34-KSL cells were identified by FITC-conjugated anti-CD34(RAM-34), PE-Cy7-conjugated anti-Sca-1, APC-conjugated anti-c-Kit, and APC-Cy7-conjugated or biotinylated anti-lineage marker antibodies. The biotinylated antibodies of interest were developed with streptavidin-PE. PE-conjugated anti-rabbit IgG was used for visualization of unconjugated antibody. To prepare cells for sorting, low-density cells were depleted of lineage marker positive cells using magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Flow cytometric analysis and sorting were performed on a MoFlo (Beckman Coulter, Fullerton, CA) or a FACS Vantage SE (BD Bioscience, San Jose, CA). Transplantation. Competitive repopulation assays with 2×10^5 whole bone marrow cells from Ly5.1/Ly5.2-F1 mice were performed in B6-Ly5.2 mice lethally irradiated with 2 doses of 4.75 Gy, delivered 4 hours or more apart. One cell or 10 cells were transplanted per recipient mouse. Peripheral blood cells from the recipient mice were analyzed at 1, 2, 3, 4, and 5 months after transplantation. After erythrocyte lysis, cells were stained with FITC-conjugated anti-Ly5.2, PE-Cy7-conjugated anti-B220, PE-conjugated anti-CD4 and -CD8, APC-conjugated anti-Mac-1 and -Gr-1, and biotinylated anti-Ly5.1 antibodies. The biotinylated antibody was developed with streptavidin-Alexa 594. Six-color flow cytometric analysis was performed using a FACS Vantage SE (BD Bioscience, San Jose, CA). Percentage chimerism was defined as (percent Ly5.1+ test donor

cells) × 100 / (percent Ly5.1+ test donor cells + percent F1 competitor cells). If chimerism was 0.3% or more at any time point after transplantation, regardless of which lineage was reconstituted, mice were considered to be reconstituted with test donor cell(s), and test donor cells were considered to contain at least one repopulating cell. Latent HSCs were defined as HSCs which exhibited 0.3% or more chimerism 12 weeks or more after transplantation. Secondary transplantation was performed by transferring 5×10^6 bone marrow cells from femora and tibiae of the primary-recipient mice into each of 3-5 lethally irradiated B6-Ly5.2 mice. Peripheral blood cells from the secondary-recipient mice were analyzed at 1, 2, 3, 4, and 5 months after transplantation.

Results:

1. Fractions in CD34-KSL cells To identify cell surface markers that distinguish HSCs with heterogeneous reconstitution behaviors, CD34-KSL cells were co-stained with 118 cell surface markers. Among them, 81 markers appeared not to be expressed and 22 markers were expressed on CD34-KSL cells. Only 15 markers showed variable expression on CD34-KSL cells that permitted us to test fractions of CD34-KSL cells to determine whether they might represent functionally distinct subsets of HSCs. Based on expression of these antigens, CD34-KSL cells were sorted into positive and negative fractions by flow cytometry for transplantation assay. In the case of CD150, CD34-KSL cells were separated into CD150high, CD150med, and CD150neg fractions. Long-term repopulating activity was detected in both positive and negative fractions in all cases, suggesting that none of these markers is particularly useful for excluding non-LTRCs from CD34-KSL cells. However, we observed particularly interesting reconstitution kinetics patterns and lineage compositions in reconstituted mice in the cases of CD150.

2. Identification of distinct LTRCs In order to compare the frequencies and functional properties of LTRCs within CD150high, CD150med, and CD150negCD34-KSL cells, single-cell transplantation was performed. Results of the three 40-mouse cohorts suggest that LTRCs are equally present among CD150high, CD150med, and CD150negCD34-KSL cells. Results of secondary transplantation differed remarkably, however. After secondary transplantation, LTRCs were detected in all of the recipient mice transplanted from primary-recipient mice that had initially received CD150highCD34-KSL cells. LTRCs were rare in secondary-recipient mice whose donors had initially received CD150med and CD150negCD34-KSL cells. In addition, CD150highCD34-KSL cells predominantly reconstituted the myeloid lineage, while CD150negCD34-KSL cells predominantly reconstituted the lymphoid lineage. With respect to in vivo reconstitution kinetics, percentages of chimerism progressively increased throughout primary and secondary transplantation in 10 of 13 cases initiated with single CD150highCD34-KSL cells. Such increment was rare in those initiated with single CD150medCD34-KSL or CD150negCD34-KSL cells. Noteworthy is that a latent type and a myeloid-limited type of LTRCs existed among CD150highCD34-KSL cells. Cells of latent type represented one out of every 10 CD150highCD34-KSL cells, and produced virtually undetectable levels of blood cells for several months after transplantation. Beginning 12 weeks or later, these LTRCs effected a low level of myeloid reconstitution. However, these cells showed progressive and robust repopulating activity in secondary-recipient mice. Reconstitution kinetics in individual secondary-recipient mice revealed that reconstitution patterns were very similar among secondary-recipient mice in each group. However, in the case of latent type LTRCs, various levels of reconstitution were observed for secondary-recipient mice in each group. Furthermore, lineage composision patterns remarkably differed among recipient mice. While only myeloid-lineage reconstitution was detected in primary-recipient mice of latent LTRCs, B- and T-lymphoid lineages became readily detectable in most secondary-recipient mice.

3. Hierarchical order in distinct LTRCs From transplantation data, we predicted that CD150high cells reside in the uppermost portion of the hematopoietic hierarchy. To assess this, we evaluated the reversibility of CD150 expression. Three cohorts of 3 mice that had been reconstituted with 10 CD150high, CD150med, or CD150negCD34-KSL cells were analyzed 8 months after transplantation. Flow cytometric analysis was performed on bone marrow cells from these mice to assess CD150 expression on reconstituted CD34-KSL cells. Test donor-derived CD150highCD34-KSL cells were detected only in the mice that received CD150high-CD34-KSL supporting our hypothesis that CD150high cells are high in rank among CD34-KSL cells.

Conclusion: HSCs are heterogeneous with regard to self-renewal potential, *in vitro* colony-forming activity, and in vivo behavior. In this study, we attempted prospective isolation of HSC subsets with distinct properties among CD34-/low c-Kit+Sca-1+lineage marker-negative (CD34-KSL) cells. CD34-KSL cells were divided, based on CD150 expression, into three fractions: CD150high, CD150med, and CD150neg cells. Compared with the other two fractions, CD150high cells were significantly enriched in HSCs with great self-renewal potential. *In vitro* colony assays revealed that decreased expression of CD150 was associated with reduced erythroblast/megakaryocyte differentiation potential. All three fractions were regenerated only from CD150high cells in recipient mice. Using single-cell transplantation studies, we found that a fraction of CD150high cells displayed latent and barely detectable myeloid engraftment in primary-recipient mice but progressive and multilineage reconstitution in secondary-recipient mice. These findings highlight the complexity and hierarchy of reconstitution capability, even among HSCs in the most primitive compartment.

Keywords: Hematopoietic Stem Cells, Hierarchy, Heterogeneity, CD150, Long-Term Marrow Reconstitution

Is-20: Adipose Tissue-Derived MSCs Are an Option to Human Hepatocytes for *In vitro* and *In vivo* Studies

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Due to limited human primary hepatocyte availability, researchers are tracking on alternative cells with a broad spectrum of metabolic capacity. These cells are generally of high interest for those working in the field of drug development or transplantation. For this purpose, several groups attempt to generate hepatocyte-like cells from various adult stem or precursor cells such as mesenchymal stem cells (MSCs), fat tissue (Ad-) or liver. Besides reduced expression of hepatocytic-markers and drug metabolizing capacity, the major drawback with this technique is limited amount cells generate in a reproducible manner. Aim of this study was to contrast metabolic capability and pluripotency of Ad-MSC with human hepatocytes. Ad-MSCs and hepatocytes were isolated from different patients, with their informed consent according to ethical guidelines of the MRI. For hepatic differentiation several supplement combinations were used and evaluated. The generated hepatocyte-like cells were stained for Glycogen (PAS), Glucose-6-phosphatase and neutral lipids (Oil Red O staining). We further investigated glucose and urea metabolism as well as several phase I and II drug metabolizing enzyme activities. Expression of pluripotency-, mesoderm- and endoderm-markers was analyzed by RT-PCR. Differentiated and non-differentiated cells were injected into the spleen of mice and tracked by immunohistochemistry. During differentiation Ad-MSCs gain the ability to accumulate glycogen and express G6P. Best results could be generated by differentiation of Ad-MSCs, after demethylation, with a supplement combination of FGF4, ITS and Nicotinamid. Glucose production expression reached 50% of non-differentiated cells. Compared to human hepatocytes differentiated cells generated 60% urea production and reached up to 100% of Phase I and II enzyme activities. Our work identified Ad-MSCs as a reliable cell source to differentiate these cells into hepatocyte-like cells which are highly reproducible and show consistent metabolic capacity. In addition the cells integrated into the liver. Hence, these cells may be used as alternative metabolic system for drug screening and toxicity studies as well as a future source for cell transplantation.

Is-21: Epigenetic Modification of Old Human Adipose - Derived MSCs Improves Hepatic Differentiation

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Objective: The aim of our present study is to establish standardized Ad-MSC-derived hepatocyte-like cells (HLCs). Preliminary results showed donor differences between young and old Ad-MSCs. We analyzed the age-related differences before and after hepatic differentiation.
Materials and Methods: Ad-MSCs were isolated from abdominal adipose tissue of young (≤45a) and old (>45a) donors according to ethical guidelines of TU Munich. Proliferation (Alamar blue assay, KI67 Staining) and expression of pluripotency markers (Oct4, Nanog, Sox2, c-Myc, KLF4) were determined. Hepatic differentiation with/without 5-azacytidine (AZA) was initiated in passage 3; afterwards enzymatic and metabolic activities of HLCs were measured.
Results: Ad-MSCs derived from old donors demonstrated less proliferation capacity than young ones. The global methylation status in young Ad-MSCs was lower than in old ones, which concurred with the expression of Nanog and Sox2 being stronger in younger donors. Ad-MSC-derived HLCs from young and old donors pretreated with AZA, demonstrated similar enzymatic and metabolic activities; in some cases they reached similar levels as seen in human hepatocytes. Ad-MSCs from old donors without AZA pretreatment showed a much lower hepatic differentiation capacity than young ones.
Conclusion: Reduced expression of Nanog and Sox2 may be a causative factor for poor proliferation of old Ad-MSCs and their consequently lower metabolic capacity after hepatic differentiation.

Is-22: ESCs Require PRC2 to Direct The Successful Reprogramming of Differentiated Cells Toward Pluripotency

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Is-23: Heterokaryon-Based Reprogramming for Pluripotency

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Is-24: Bone Marrow as Ideal Microenvironment for Human Islet Transplantation to Treat Type 1 Diabetes (Clinical Trials.gov Identifier: NCT01345227)

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Objective: The goal of this study is to evaluate safety and efficacy of bone marrow (BM) as site for pancreatic islet transplantation in humans. Our hypothesis is that BM represents a better site than liver (currently the location of choice for this procedure) thanks to its potential capacity to favor islet engraftment in face of a similar easiness of access. To address our hypothesis we propose herein a randomized phase I/II trial to compare BM and liver as sites for islet transplantation in patients with type 1 diabetes (T1D).
Materials and Methods: A pilot study has been approved by the Italian Regulatory Agencies in August 2009. We were permitted to perform single intra BM islet infusion at the level of the iliac crest in patients having contraindications for intraportal infusion. Until now 8 patients were recruited: 4 Islet Auto Transplant (IAT, #1, #5, #6, #8), 2 Islet After Kidney (IAK, #2, #4), 2 Islet Transplant Alone (#3, #7).
Results: A needle for BM aspiration (14 G) was inserted into the superior-posterior iliac crest and islet suspension (1:2.5; tissue:Ringer's Lactate solution) was infused (median volume 8 ml; min-max: 2.5-20 ml). The entire intraBM-injection procedure lasted 8-15 minutes from the beginning of anaesthesia (short propofol sedation). No complications occurred during the islet infusion. Patient #1 died at day 5 for IAT unrelated fatal bleeding. All the other patients are alive without any intraBM-Tx related complication (median follow-up 368 days). In all recipients islets engrafted successfully as shown by the presence of postTx circulating C-peptide >0.5 ng/ml and by the presence of insulin producing cells and/or molecular markers of endocrine tissue at bioptic follow up. IAT maintained Tx function during the time, while IAK and ITA lost it with timing similar to patients intraportally infused and treated with the same immunosuppression
Conclusion: Intra BM islet infusion is a safe and feasible. Efficacy needs to be tested in a randomized, phase II trial in which patients will be randomized to receive islets into either the liver or the BM.
***Keywords*:** Beta Cell Replacement, Diabetes, Human

Is-25: Regulatory Stem Cell and Allograft Tolerance

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Is-26: Induction of Pluripotency: 20 Years of Research

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The mammalian germline comprises the inner cell mass (ICM) and epiblast, containing pluripotent cells, and the germ cell lineage, hosting unipotent cells. Embryonic stem (ES) cells, derived from preimplantation embryos, comprise two cell types with divergent states of pluripotency. Similarly, epiblast stem cells (EpiSCs), derived from postimplantantion embryos, comprise cells of early and late pregastrulation embryos.
The ultimate goal of cell and developmental biology is to program cells at will. The first step in converting any given cell type into another cell type is to achieve a pluripotent stem cell state that resembles that of ES cells. Somatic cells need exogenous transcription factors to achieve pluripotency. Reprogramming of mouse and human somatic cells into pluripotent stem cells, termed induced pluripotent stem (iPS) cells, was first described in 2006 using fibroblasts (somatic cells) and initially required the virally-expressed transcription factor quartet of Oct4, Sox2, c-Myc, and Klf4. Later, we reported that Oct4 alone is sufficient to directly reprogram adult mouse and human fetal neural stem cells (NSCs) into iPS cells, showing that Oct4 plays a crucial role in the reprogramming process. We recently showed that induced EpiSCs (iEpiSCs) can be obtained by directly reprogramming somatic cells with the quartet under EpiSC culture conditions.
In contrast to somatic cells, primordial germ cells (PGCs) were first induced to pluripotency 20 years ago by the mere modulation of the culture conditions. We recently converted adult germline stem cells (GSCs) into germline-derived pluripotent stem (gPS) cells. GSCs are unipotent testis cells capable of not only self-renewing, but also giving rise to sperm. Like ES cells, GSCs exhibit significant levels of Oct4 and Klf4, but low levels Sox2 and c-Myc.
To better understand the reprogramming process, we sought to identify factors that mediate reprogramming with higher efficiency. We established an assay based on *Oct4* reactivation to screen nuclear fractions from extracts of pluripotent cells. BAF chromatin remodeling complexes containing the Brg1 protein enhance the efficiency of quartet-mediated reprogramming of somatic cells to pluripotency. As knockdown of Brg1 leads to differentiation of ES cells, we investigated the early effect of Brg1 knockdown on the expression of key pluripotency factors via RNA interference. We show that Brg1 knockdown leads to immediate Sox2 downregulation, Nanog upregulation, and *Oct4* upregulation, the latter perhaps triggering ES cell differentiation. Our data suggest that Brg1 plays an important role in regulating the expression of Oct4, a factor instrumental in maintaining cellular pluripotency.

Is-27: The Potential of Induced Pluripotent Stem Cells in Development and Regenerative Medicine

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The reprogramming of mouse and human somatic cells into pluripotent stem cells, designated as induced pluripotent stem (iPS) cells, was first successfully achieved using fibroblasts as the starting population in 2006 by Kazutoshi Takahashi and Shinya Yamanaka. Initially, the introduction of the virally-expressed transcription factor quartet of Oct4, Sox2, c-Myc, and Klf4 was a prerequisite to reprogramming. Subsequently, we reported that *Oct4* alone is sufficient for the direct reprogramming of adult mouse and human fetal neural stem cells (NSCs) to iPS cells. Alternate methods of inducing mouse pluripotent stem cells involve the use of recombinant proteins and when certain adult unipotent stem cells are used as the starting cell population, introduction of extraneous transcription factors are not needed.
Reprogramming adult cells to pluripotency has uncovered exciting new areas in basic and applied research. In my presentation, I will introduce the following three areas as well as discuss the basic principles underlying the induction of pluripotency:
• Potential use of reprogrammed cells capable of supporting cellular transplantation
• Development of mouse models for human diseases
• Generation of iPS cells and screening of potential new drug candidates.

Is-28: Direct and Complete Neural Induction of Human ES and iPS Using Small Molecules

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Pluripotent stem cells can self-renew indefinitely under appropriate cell culture conditions and generate all somatic cell types of the human body. Although significant progress has been made with regard to understanding the molecular circuitry of core transcription factors and cell signaling pathways that maintain the pluripotent state, our knowledge of controlled lineage specification and cellular differentiation is still very limited. To elucidate neural lineage entry, we set out to define a method that allows controlled and robust neural induction. Based on the hypothesis that neural induction may come under control when manipulating the signal transduction pathways that promote pluripotency and/or mesendoderm differentiation, we tested various small molecules for their potency to enhance neural conversion. This presentation will discuss how human pluripotent stem cells (ES and iPS) can be rapidly and completely coaxed into neuroectoderm under feeder-free monolayer conditions. The resultant pure populations of definitive neural stem cells (97% PAX6+/PLZF+/Nestin+) can then be differentiated into an array of specialized neuronal and glial phenotypes confirming their broad multipotent potential; thereby firmly establishing this neural induction method as a platform to study human neural development *in vitro*. Moreover, using a proteomics approach we searched for autocrine factors that may contribute to early fate choice. The identification and functional characterization of an autocrine factor with strong biological effects suggests that the neuralization process is controlled by an "instructive" rather than a "default" mechanism.

Is-29: Protein Phosphorylation Signatures Define "Stemness" in Human Pluripotency and Neural Multipotency

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Understanding defined developmental and cellular states at a systems biology level is one of the great challenges in stem cell biology. Pluripotent stem cells can generate all somatic cell types of an organism, while multipotent stem cells are more restricted in their developmental potential and typically give rise to cell types of a given lineage or organ. Previous studies searched for "stemness" signatures using microarray technology (Ivanova et al. 2002; Ramalho-Santos et al. 2002) but potential "stemness" transcripts showed little overlap and reproducibility across different laboratories (Vogel, 2003; Pyle et al. 2004). Using pluripotent human embryonic stem cells as a model system, we addressed if different "stemness" states can be better defined by delineating in-depth comparative proteomic and phosphoproteomic profiles. Multidimensional liquid chromatography (LC) combined with tandem mass spectrometry (MS/MS) provides unbiased discovery of (phospho) proteins. To this end, we established optimized cell culture conditions that maintain the feeder-free growth of pluripotent stem cells (OCT4+/PAX6-) and upon neural induction generate highly pure populations of multipotent neural stem cells (OCT4-/PAX6+). Here, we present the largest comparative (phospho)proteomic dataset generated to date (12,904 proteins; 59, 680 non-redundant phosphorylation sites). Biological insights include the novel definition of the pluripotent and multipotent state by phosphorylated transcription factors, proteome-wide differential protein abundance, and distinct posttranslational modifications. Systems-level analysis of the components and modifiers of the

human epigenome (e.g. Polycomb and Trithorax group, histone variants, chromatin remodellers) revealed that dynamic changes of protein abundance and differential phosphorylation correlated with predicted chromatin states in pluripotent and differentiated cells. The quantitative dataset allowed precise and detailed comparisons of these different "stemness" states revealing unique cell type-specific signatures on the posttranslational level, not detectable with microarray technology. Together, these findings shed new light on the proteome and phosphoproteome of two distinct cellular states and capture the dynamic transition from pluripotency to neural lineage entry as a rich resource for future studies.

Stem Cells

Os-1: Influences of PPARγ and PEP on Proliferation and Differentiation of Mouse Embryonic Stem Cells

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Os-2: Optimized Zona-Free Method of Somatic Cell Nuclear Transfer in The Goat

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It is widely accepted that goat (*Capra hircus*) is the ideal species for transgenic production of recombinant proteins. Therefore, it is expected that the demand for production of transgenic herds of goat with the use of a simple, fast, and efficient method of cloning will increase. Accordingly, this study was carried out to develop an improved zona-free method of goat somatic cell nuclear transfer (SCNT) that has both ease of operation and efficiency. The main steps involved were:
1. Optimization of *in vitro* oocyte maturation
2. Parthenogenetic activation of zona-free oocytes
3. SCNT of zona-free anaphase IItelophase II (AII-TII) oocytes that subverted the need for long term UV-exposure of the oocytes
4. *In vitro* culture of groups of cloned embryos in wells in a highly efficient continuous serum-free embryo medium to the blastocyst stage before transfer to the recipients. To our knowledge, this is the first report of successful live and survived birth of cloned and transgenic offspring through a whole procedure of in vitro oocyte maturation and embryo development to the blastocyst stage, and in this study the *in vitro* efficiencies of cloned and transgenic embryo production were higher than the available reports.

Keywords: Somatic Cell Nuclear Transfer, Blastocyst, Transgenic Goat

Os-3: Isolation and Characterization of Cancer Stem Like Cell Based on Expression of CD44 in Prostatic Cancer Cell; An Experience on DU145 Cell Line

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Objective: Cancer stem cells (CSC) were suggested to be present in human prostate cancer as a small population of distinct cells. They are capable of tumor initiation and self-renewal to recreate entire tumor. So they are attractive targets for cancer therapy. The aim of this study is the isolation of CD44 positive cells and determines stemness properties of them.
Materials and Methods: DU145 cells were cultured on complete medium and sorted up on expression of CD44 using BD FACS Aria cell sorter. Colony formation potential, proliferation rate, side population and serum free assay were performed on sorted cells. the expression of proposed cancer stem cell markers " CD133, CD44, CD29, α2β1 integrine" was evaluated by FACS and stemness genes "OCT4, Sox2 and Nanog" by RT-PCR.
Results : The number of cells and large colonies was higher in CD44+ cells than CD44- cells ($3.1 \times 10^5 \pm 5.3 \times 10^4$ vs. $6.3 \times 10^4 \pm 1.5 \times 10^4$ and 11 ± 1.15 vs. 9 ± 3.5 respectively) after 14 days culture. CD44+ cells were proliferated on serum free medium, however the CD44- growth arrest in the same medium. The expression of CD133and β1 integrine was absent and α2 was in low amount in CD44- compare to CD44+ cells. The stemness genes "OCT4 and Sox2" were expressed in positive fraction. Moreover 0.1% of CD44+ fraction shows SP properties.
Conclusion: Our result suggested that cells which co expresses CD44 and β2 integrin are relative candidate for Prostate cancer stem like cells. However the tumorogenicity of CD44+ β2 integrin+ must be estimated in NOD-SCID mice.

Keywords: Prostate Cancer, Cancer Stem Cells, CD44, Cell Sorting

Os-4: Co-Transplantation of Human Embryonic Stem Cell-Derived Neural Progenitors along with Schwann Cells in a Rat Spinal Cord Contusion Injury Model Elicits a Distinct Neurogenesis and Functional Recovery

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Os-5: Neural Fate of Human Embryonic Stem Cells Derived Neural Precursor Cells Following Chick Embryonic Somites and Notochord Co-Culture

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Objective: Regarding to limitations of *in vivo* study in human nervous system development, it is necessary to design an *in vitro* model to evaluate the *in vivo* effect of surrounding tissues on neurogenesis and regional identity of human neural plate. On the other hand the neural patterning role/roles of somites and notochords in regional compartments of neural tube along rostro-caudal and dorso-ventral axes, especially in humans, remain elusive.
Materials and Methods: Rostral neural progenitors (NPs) were initially generated from adherent human embryonic stem cells (hESCs) in a defined condition and characterized. Then, to find the role of somites (S) and notochords (N) in rostro-caudal (RC) and dorso-ventral (DV) patterning of neuronal cells, NPs were co-cultured with microencapsulated chicken somites or notochords in alginate beads.
Results: It was revealed that notochord induced spinal cord ventral brachiothoracic identity with expression of IRX3, PAX6, NKX6.1, NKX2.2, EVX1, EN1, CHX10, and HOXC8 and motoneuron makers, HB9, ISL1, ISL2, LHX1, and LHX3 in addition to its proliferation effect on NPs. We observed a synergic effect on motoneuron induction particularly lateral motor column subtype when S and N were used together. Moreover, S induced hindbrain identity with expression of HOXB4 in differentiated neuronal cells from NPs.
Conclusion: Our results indicated that highly enriched NPs can be generated in an adherent and defined system from hESCs. Moreover, S and N tissues were highly influenced neuronal differentiation in point of proliferation, neurogenesis, RC and DV patterning. These results indicate a very simple and efficient protocol to mimic *in vivo* events for human neural development *in vitro* which is important in the context of cellular replacement therapies.
Keywords: Human Embryonic Stem Cells, Chicken, Co-Culture, Neural Progenitors, Neural Patterning, Notochord, Somite

Os-6: Functional Genomics Reveals a BMP Driven Mesenchymal-to-Epithelial Transition in the Initiation of Somatic Cell Reprogramming

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Objective: Previous studies seeking genes that regulate embryonic stem cell maintenance resulted in identification of four transcription factors that induce somatic cell reprogramming. Despite the advancement made in derivation and characterization of induced pluripotent stem (iPS) cells, little is known of the mechanisms that underlie reprogramming. Therefore a more comprehensive approach to identify other key regulatory pathways essential for reprogramming is needed.
Materials and Methods: To identify regulatory mechanisms governing iPS cell generation; first we utilized temporal expression profiling of reprogramming cells throughout reprogramming. Then, we established conditions for a systematic RNAi screen to evaluate the function of a large set of genes during reprogramming. The resulting dataset provides the foundation for dissecting the complex signaling networks mediating the reprogramming process. To conduct these genome-scale screens, we took advantage of a mouse embryonic fibroblast cell line with a unique transgene delivery system. In this system, a doxycycline inducible promoter is used to regulate expression of four reprogramming factors, Oct4, Klf4, c-Myc and Sox2, utilizing a piggybac transposon-transposase delivery system. Using this system driving secondary iPS cells, we performed an RNAi screen on a custom library to knockdown 4010 genes individually. In this assay, the effect of specific gene knockdown on cellular reprogramming was assessed. Using this strategy, we have been able to identify both activators and repressors of reprogramming.
Results: Temporal analysis of gene expression revealed that reprogramming is a multi-step process that is characterized by Initiation, Maturation and Stabilization phases. Functional analysis by systematic RNAi screening further uncovered a key role for BMP signaling and the induction of mesenchymal-to-epithelial transition (MET) during the Initiation phase. We show this is linked to BMP-dependent induction of miR-205 and the miR-200 family of microRNAs that are key

regulators of MET.
Conclusion: These studies thus define a multi-step mechanism that incorporates a BMP-miRNA-MET axis during somatic cell reprogramming.
Keywords: Reprogramming, Microarray Analysis, RNAi screen, BMP Signalling, Mesenchymal-to-Epithelial Transition

Os-7: Characterisation of PLGA Scaffold in Presence of Primary Limbal Epithelial Cell for Replacement of Human Amniotic Membrane for Delivering Cells to The Cornea

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Objective: The aim of our research is to make a synthetic biodegradable membrane to replace the human amniotic membrane which is currently used for delivering cultured limbal stem cells to the cornea.
Materials and Methods: To achieve this we are developing biodegradable Poly DL-lactic-co-glycolic (PLGA; Sigma Aldrich, with a 50:50 ratio of PLA and PGA) scaffolds using electrospinning. PLGA is one of the most widely used biodegradable polymers since it is biocompatible and its degradation rate can be easily controlled by varying the copolymer ratio of lactic to glycolic acid. Preliminary studies investigated the wettability of these electrospun scaffolds by measuring contact angles of scaffolds in medium, PBS and water. The rate of degradation of the PLGA scaffolds was also investigated *in vitro*. Rabbit limbal epithelial cells isolated from limbus where cornea stem cells are present and were seeded on scaffolds for up to 8 weeks via stainless steel rings which were also used to prevent scaffold contraction. The scaffolds were examined using light microscopy and scanning electron microscopy (SEM).
Results: The results showed that the dry PLGA 50:50 scaffolds have an average contact angle of 106 degrees but this angle fell rapidly when they were immersed in liquid. Scaffolds in media showed significantly faster wettability compared to scaffolds in PBS and water. This indicates that contact angles are a sensitive method for assessing wettability of scaffolds. The preliminary results of *in vitro* degradation of PLGA scaffolds with initial fibre diameter of 5µm show that the degradation rate of PLGA in the presence of cells is faster than that of the same scaffold with media only.
Conclusion: The results indicate that scaffolds in media showed faster wettability compared to scaffolds in PBS and water. Degradation study of PLGA scaffolds show faster rate of degradation in the presence of cells.
Keywords: PLGA scaffold, Degradation, Wettability

Os-8: Tumor Microenvironment and Tissue Resident Stem Cell Interactions: A Tool for Targeting Cancer Stem Cells

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Objective: Tumor microenvironment (TM) is comprised of cancer stem cells, tumor cells as well as a wide network of stromal and vascular cells participating in the cellular and molecular events necessary for invasion and metastasis. TM is commonly known as site of inflammation and cytokine production. Tresident stem cells (SCs) eliciting regenerative properties are known to home to sites of inflammation, tissue injury and tumor. Considering the tumor tropic property of SCs, the interaction between breast cancer (BC) and SCs needs further investigation. In this study, we characterized the molecular basis of local SCs trafficking to BC cells.
Materials and Methods: In this study we employed several *in vivo* and *in vitro* molecular biology techniques including transwell migration assay, invasion assay, q2PCR array, microRNA array, flowcytometry, and protein detection.
Results: Further analyzing this TM and ASC interaction identified a tumor tropic subset of SCs reside in breast tissue, which displays significant migratory activity. Characterizations of the tumor tropic migratory subset of SCs indicate the basic function of TM on anchoring SCs. This anchoring effect results in recruitment and re-programming SCs in functional level. Profiling the tumor tropic migratory subpopulation of SCs showed an increased expression of adhesion molecules and genes responsible for cell growth and proliferation. TM caused up regulation of extracellular matrix genes. Additionally, expression of metastasis associated genes (CD82 and CST7), receptors for growth and proliferation (MET), and transcription factors (TCF20) were up regulated in migratory SCs. Further analysis showed a perturbed expression of miRNAs in migratory subset of SCs.
Conclusion: TM's secretory factors can activate the migration of host cells, both near to and far to the primary tumor site, as well as promoting the exodus of cells to distant tissues. Thus, the migration of stem/progenitor cells (SCs) and tumor cells among specialized microenvironments takes place throughout tumor and metastatic progression, providing evidence for the systemic nature of a malignancy. One important effect of TM is on the SCs which reside in surrounding tissue. It has been recently noted that breast tissue resident SCs

and their niches interact with breast cancer TM. The knowledge obtained from these studies is an important piece in understanding tumor-tissue interaction. Investigations of the tumor–stromal and stromal–stromal cross-talk involved in cellular migration in cancer can lead to novel therapeutic strategies for targeting cancer stem cells as well as tumor cells.

Keywords: Tumor Microenvironment, Tissue Resident Stem Cells, Breast Cancer, Cell Migration, Cancer Stem Cell

Os-9: Xenotransplantation of Cryopreserved Human Ovarian Tissue into Murine Back Muscle

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Objective: Ovarian tissue (OT) cryopreservation and transplantation are options for fertility preservation in young female cancer patients.
Materials and Methods: We investigated xenotransplantation of human OT into back muscle (B) of severe combined immunodeficiency mice. OT follicle content was evaluated by stereomicroscopy and pretransplantation. Xenograft survival, follicular development (with/without FSH administration), apoptosis and vascularization were compared in B- versus K-site (under the kidney capsule) several times after grafting using histology, immunohistochemistry and magnetic resonance imaging. *In vitro* maturation (IVM) was also performed.
Results: Anastomoses which developed from existing human and invading murine vessels were seen in OT at both sites, but angiogenesis was more prominent at the B- than K-site ($p<0.001$). Vascularization and follicle size were correlated in the B-group (Spearman's coefficient 0.73; $p<0.001$). FSH increased early (8 days) micro-vessel formation in B but not in K grafts ($p<0.0001$), versus no FSH). B-site grafts showed a better histological morphology and survival ($p<0.0084$), formation of larger antral follicles ($p<0.005$), more metaphase-II (MII) oocytes, growing follicles ($p<0.028$) and slightly fewer apoptotic follicles than K grafts. One MI oocyte from B underwent IVM and reached MII stage next day.
Conclusion: To our knowledge, this is the first report of MII and IVM–MII oocytes obtained from B xenografts. We report the largest oval-shaped antral follicles containing an MII oocyte obtained after OT xenotransplantation to date. Xenografting in the mouse B should be further explored as a method for human OT transplantation.

Keywords: Xenotransplantation, Ovary Cryopreservation, Human Vary, Muscle, Metaphase II Oocyte

Os-10: Co-transplantation of hESC-NPs and SCs in a Rat Spinal Cord Contusion Injury Model Elicits a Distinct Neurogenesis and Functional Recovery

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Co-transplantation of neural progenitors (NPs) with Schwann cells (SCs) might be a way to overcome low rate of neuronal differentiation of NPs following transplantation in spinal cord injury (SCI) and the improvement of locomotor recovery. In this study, we investigated human embryonic stem cells derived neural progenitor cell (hESC-NPs) potential for neuronal differentiation and functional recovery when co-cultured with adult rat purified SCs in vitro and co-transplanted in a rat acute model of contused SCI. Co-cultivation results revealed that the presence of SCs provided a consistent status for hESC-NPs and recharged their neural differentiation toward a predominantly neuronal fate. Following transplantation, a significant functional recovery was observed in all engrafted groups (NPs, SCs, NPs+SCs) relative to the vehicle and control groups. We also observed that animals receiving co-transplants instituted a better state as assessed with the BBB functional test. Immunohistofluorescence evaluation five weeks after transplantation showed invigorated neuronal differentiation and limited proliferation in the co-transplanted group when compared to the individual hESC-NPs grafted group. These findings have demonstrated that the co-transplantation of SCs with hESC-NPs could offer a synergistic effect, promoting neuronal differentiation and functional recovery.

Keywords: Rat Schwann Cell, Human Neural Progenitor, Co-Culture, Co-Transplantation, Differentiation, Spinal Cord Injury

Animal Biotechnology

Ps-1: Time Dependent Effect of Post Warming Interval on Microtubule Organization, Meiotic Status, and Parthenogenetic Activation of Vitrified *In vitro* Matured Sheep Oocytes

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Objective: It is a common practice to rest vitrified-warmed matured oocytes for 1-3 hour(s), as a treatment to recover spindle and cytoskeleton, before commencing a further treatment. Vitrified-warmed matured oocytes, however, are very sensitive and may resume meiosis spontaneously during this recommended rest time. Therefore, the aim of this study was to assess spindle and chromosome status as well as developmental competence of vitrified *in vitro* matured parthenogenetically activated sheep oocytes.
Materials and Methods: Vitrified-warmed oocytes along with their corresponding non-vitrified oocytes activated parthenogenetically, either 0 hour (immediately) or 2 hours (delayed) after warming.
Results: There was no significant effect of post-warming interval on the proportion of degenerated oocytes. Evaluation of chromosomes and meiotic spindle configuration showed that 11.11% of oocytes in the immediate group and 8.82% of oocytes in the delayed group had normal chromosomal alignment on well-structured spindles, compared to non-vitrified group (79.41%). Meanwhile, majority of the chromosomal abnormalities in the immediate and delayed groups were categorized as absent (unobservable) (77.78%) and anapest II (70.59%), respectively. Oocytes in immediately activated group showed significantly higher blastocyst rate (28.86%) compared to delayed activated group (16.47%).
Conclusion: The results suggest that post warming interval may have important consequence on meiotic progression and parthenogenetic activation of vitrified oocytes. In sheep, it appears that chemical activation without having to await microtubule reorganization improves embryonic development.
Keywords: Cryopreservation, Oocyte, Meiosis Resumption, Developmental Competence

Ps-2: The Effect of Oocyte Activation with DTT on Development of Ovine Embryos in ICSI

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Objective: The objective of the current study was to determine the effect of activation of injected oocytes with dithiothreitol on development of ovine embryos derived from *in vitro*-matured oocytes and intracytoplasmic sperm.The injected oocytes with non-treated sperm were activated with 1) ionomycin (Io) + 6-dimethylaminopurine (6-DMAP) 2) DTT, 3) DTT+ ionomycin(I)+6-DMAP.
Materials and Methods: Within 1 hour after injection, the injected oocytes (ICSI groups) were activated as the following groups: I) Exposure to 5 µM ionomycin in H-SOF with 1% FBS for 5 minutes, culture in IVF-SOF for 3 hours to allow extrusion of the second polar body, and then exposure to 1.9 mM 6-DMAP prepared in H-SOF at 39 ºC, 5% CO_2 in air for 3 hours; II) Exposure to 2 mM DTT in H-SOF at 38.5ºC for 20 minutes and washed twice (10 minutes each) in H-SOF; III) Exposure to 2 mM DTT in H-SOF at 38.5ºC for 20 minutes and washed twice (10 minutes each) in H-SOF accomplished with ionomycin followed 3 hours later by 6-DMAP. After activation, oocytes were washed in HSOF and placed into 20 µl drops of IVC.
Results: The cleavage rates in those activated with DTT (n=78) was lower ($p < 0.05$) than those activated with either ionomycin (Io) + 6-dimethylaminopurine (6-DMAP) (n=277) or DTT+I+6-DMAP. (n=74) (30.8 % vs. 78.3% and 89.2% respectively) and also the blastocyst rates in those activated with DTT was lower ($p < 0.05$) than those activated with either ionomycin (Io) + 6-dimethylaminopurine (6-DMAP) or DTT+I+6-DMAP (0% vs. 17% and 9.5% respectively).
Conclusion: Moreover, DTT was not effective on oocyte activation compared with Io+6-DMAP after ICSI.
Keywords: ICSI, Dithiothreitol, Ovine, Activation

Ps-3: Conservation Cloning of Esfahan Mouflon

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Objective: Among wide range of bio-conservational strategies envisaged, recent accomplishments in the field of somatic cell nuclear transfer (SCNT) holds considerable promise due to its unique potential to decelerate or prevent rapid loss of animal genetic resources, and even to revive extinct species. This study was undertaken to investigate whether domestic sheep *in vitro* matured and enucleated cytoplast can

support reprogramming and *in vitro* development of cryo-banked somatic cell nuclei of Ovis orientalis isphahanica (Esfahan Mouflon), a vulnerable species classified by IUCN, into cloned blastocysts and whether these embryos can implant in the uterus of domestic sheep and develop to viable cloned offspring.

Materials and Methods: Cryo-banked fibroblast cell lines of one male mouflon from a genome resource bank and a domestic sheep were prepared and used for karyotyping analysis. Prior to SCNT, fibroblast donor cells were serum starved for 5 days. Using zona-free SCNT technique, *in vitro* matured and enucleated domestic sheep oocytes were reconstructed with nuclei donor cells of mouflon and domestic sheep and their competence for *in vitro* development to the cloned blastocyst was compared. The cloned mouflon blastocysts were then transplanted in the uterus of the synchronized domestic sheep.

Results: Karyotyping analysis confirmed that cryo-banked somatic cells of Esfahan mouflon had the correct number of diploid chromosomes (2n=54). Evaluation of 907 activated reconstructs [Esfahan mouflon (n=667), domestic sheep (n=240)] revealed no significant difference in overall blastocyst development (7.6 ± 0.5% vs. 9.3 ± 0.5%, respectively). After transfer of 12 cloned Esfahan mouflon blastocysts into 5 domestic sheep recipients, 2 (40%) pregnancies were established and both (100%) sustained until cesarean section at days 147 and 150 of pregnancy, which both culminated in the live birth of cloned Esfahan mouflon lambs.

Conclusion: The newborns did not survive and died soon after birth. Karyotype and genetic analyses confirmed that both clones 1) had correct diploid chromosome number (2n=54), and 2) were genetically identical to each other and to their original cell donor. This study highlights the importance of conservation cloning using closely related abundant alternates.

Keywords: Vulnerable, Esfahan Mouflon, Conservation Cloning

Ps-4: Cloning and Expression of PhiC31 Integrase cDNA in Escherichia coli and Test of its *In vitro* Activity Using an Intra-Molecular Assay Vector

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Objective: Gene therapy and creation of transgenic animals are two main areas of research which is growing too fast recently. In these fields, there is a serious need for integration into safe locations viewing not to destroy the other genes or important sites in the target genome. Applying site specific integrase such as phage phiC31 is one of the best tools for this approach. Phage phiC31 performs recombination between some specific attachment sites on the phage DNA and its bacterial host genome, known as attP and attB, respectively. There are lots of reports which state that there are some site-specific regions in the mammalian genomes similar to attP. These sites are known as pseudo attP. There is possibility of producing transgenic animals and performing safer gene therapy studies by using phiC31 integrase to integrate the gene of interest in to pseudo attP. The objective of the present study is to clone the phiC31 integrase cDNA in a bacterial expression vector and to express it in E.coli. After purification of integrase protein, we tried to test its *in vitro* activity by a molecular assay vector. This molecular assay vector contains both attP and attB sites in correct orientations. There is a specific fragment between these two sites in this vector. If phiC31 integrase acts properly, it can do a recombination in this molecular assay vector leading to delete the specific DNA fragment located between attP and attB in the vector. This deletion can be determined easily by PCR.

Materials and Methods: cDNA of phiC31 was amplified and cloned in an bacterial expression vector. The construct was expressed in E.coli. BL21 (DE3). Produced integrase was purified under non-denaturing condition using Ni2+ agarose columns. Its concentration was determined by Bradford assay and the related size was confirmed by SDS-PAGE analysis. The purified protein was concentrated and stored at -70◦C. The molecular assay plasmid was incubated with 1µg purified integrase in a reaction buffer for 1 hour at 37°C. A portion of this reaction was transferred to a PCR reaction mixture as template in order to investigate the desired deletion showing proper activity of the produced integrase.

Results: SDS-PAGE analysis of the purified phiC31 integrase illustrates the expected size of the protein (65 kDa). Moreover, amplification of a 401-bp band confirmed the correct functionality of purified phiC31 integrase.

Conclusion: The results of this study indicated that the purified integrase has a great potential of *in vitro* site-specific integration. This protein could be useful as a means of genetic engineering in some studies such as protein microinjection in oocyte in the aim of creation of transgenic animal or gene therapy.

Keywords: phiC31 Integrase, Site Specific Integration, attP, attB

Ps-5: Ovine Sperm Pretreatment on Subsequent Development of Injected Oocytes in ICSI Procedure

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Objective: This study was design to determine the effects of various methods of sperm pretreatment on subsequent developmental potential of *in vitro*-matured sheep oocytes injected with pretreated sperm.
Materials and Methods: Prior to intracytoplasmic sperm injection (ICSI), the sperm were treated in: I) H-SOF supplemented with either 0.1% sodium dodecyl sulfate (SDS), II) 5mM dithiothreitol (DTT), III) 0.1 %SDS + 2mMDTT for 30 minutes and IV) two times freeze/thaw procedures. The injected oocytes received no treatment served as control. All injected oocyte were artificially activated with ionomycin (Io) + 6-dimethylaminopurine (6-DMAP).
Results: There were no significant difference between groups I, II, III, IV, and control in the rates of cleavage (82.5%, 85.7%, 87%, 74.5%, and 78.3%, respectively), and blastocyst formation (14.4%, 16.6%, 13.2%, 18.1% and 17%, respectively). No significant differences in blastocyst cell numbers (Inner cell mass, Trophectoderm and total cells) were observed among groups.
Conclusion: Pretreatment of sperm with SDS or DTT alone or in combination as well as freeze/thaw procedure has no significant effect on subsequent development of resulting sheep embryos.
Keywords: ICSI, Dithiothreitol, Ovine, Development

Stem Cell

Ps-6: Prevalence of Abnormal Cervical Cytology Among Subfertile Saudi Women

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Objective: Since cervical cancer is reportedly the seventh most frequent cancer in women in Saudi Arabia and the eighth most frequent cancer among women aged between 15 and 44 years, we wanted to determine the prevalence of abnormal cervical cytology among subfertile women attending the reproductive medicine unit of a tertiary care center in Saudi Arabia.
Materials and Methods: This was a retrospective, cross-sectional, hospital-based study. A Pap smear was done for 241 of 493 (48.9%) subfertile women from January 2008 through February 2009.
Results: The Pap smear was normal in 166 of 241 patients (67.9%), abnormal in 71 (29.5%), and unsatisfactory for evaluation in 4 (1.7%). According to the revised Bethesda system, epithelial cell abnormality was found in 7 (2.9%), inflammation in 55 (22.8%), and infection in 9 (3.7%) patients. Epithelial cell abnormalities were further classified as atypical squamous cells of undetermined significance (ASC-US) (n=3, 42.8%), atypical squamous cells of high grade (ASC-H) (n=1, 14.3%), low-grade squamous intraepithelial lesion (LSIL) (n=2, 28.5%), and glandular cell abnormalities (AGS) (n=1, 14.3%).
Conclusion: The high prevalence of abnormal cervical cytology in our subfertile women accentuates the need for screening in patients eligible for *in vitro* fertilization. In addition, a well-organized screening program for cervical cell abnormalities at the national level should be implemented to allow identification of subfertile women at risk so that potentially life-saving measures can be undertaken early.
Keywords: Cervical Cytology, Epithelial Cell Abnormality, Subfertility, Screening, Cancer

Ps-7: Effect of Topography on Stem Cells

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Objective: Topographical parameters are important to guide stem cell behaviour, including geometry, size, lateral spacing and surface chemistry, which have been reported to affect the adhesion, growth, proliferation, and differentiation of stem cells. Nanostuctures on biomaterials provides a useful tool for guiding diferentiation, as the features are more durable than surface chemistry and can be modified in size and shape to suit the desired application. The underlying mechanism of how topography influences stem cells remains unknown. Nevertheless, the advance of nanotechnology has enabled the fabrication of synthetic topographies with different materials, shapes and sizes, allowing systematic studies of the underlying mechanism. Here we demonstrate the importance of nanoscale surface topography as a crucial factor to control adhesion and differentiation of TERA 2 embryonic stem cells. Since orientation and symmetry may also be important, we have examined how the geometry of arrangement of zinc oxide nanotubes on a substratum affects the differentiation of stem cells growing on them under simulated in vitro conditions.
Materials and Methods: TERA 2 stem cells re-enhanced from -80°C to 37°C in culture flask.
Results: The results clearly show that these architectures caused differential guidance for the filopodia (extensions) of stem cells. Cells adhered far less to vertical ZnO nanorods than to horizontal ZnO. The few cells that adhered to vertical ZnO nanorods were rounded and not viable compared to horizontal ZnO. Cells that initially

adhered to the vertical nanorods were unable to spread. This suggests that it is the lack of initial attachment and spreading that causes premature cell death.
Conclusion: The ZnO nanorods are useful to affect the behavior of stem cells and, potentially their differentiation. Altering the orientation of nanotubular zinc oxide on the surface revealed changes in stem cell behaviour, offering a promising nanotechnology-based route for directed tissue engineering with excellent controllable mechanical properties.
Keywords: Stem Cells, Nanotechnology, Topography, Differentiation, Nanotextures

Ps-8: Expression Pattern of REST in hESCs and hiPSCs Undergoing Neural Differentiation

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Objective: The neuronal repressor REST (RE1-silencing transcription factor) is expressed at high levels in embryonic stem cells (ESC) with a critical role in self-renewal and pluripotency signaling network of these cells. REST is an essential element for brain development and also neuronal differentiation of ESC *in vitro*. Although REST is a well-known regulatory element in embryonic stem cells, but according to our knowledge, it has not been evaluated in induced pluripotent stem cell (iPSC) till now.
Materials and Methods: The quantitative expression of REST in each step was evaluated by real-time PCR and the presence of REST was showed by immunofluorescent assay.
Results: Q-PCR analysis showed that the expression level of REST decreased significantly during differentiation of hiPSCs to neural precursor cells, and remained stable until neuronal differentiation. immunofluorescent data revealed the both nuclear as cytoplasmic presence of REST in hiPSCs as well as NSCs, while in the maturated neurons it was only detectable in the cytoplasm.
Conclusion: Our findings show a distinct expression pattern for REST during hESCs and hiPSCs neural differentiation. This study opens a new window for further experiments in this field to receive efficient differentiation of neuronal cells.
Keywords: REST, hESC, hiPSC, NSC, Neural Cells

Ps-9: Developing a Flowcytometry-Based Protocol for Detection of Dental Pulp Stem Cells

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Objective: Colony-forming unit-fibroblast (CFU-F) assays have historically been used for the enumeration of rare dental pulp stem cells (DPSCs). However, this protocol needs to isolate and at least 14 days culturing techniques. Low time consuming method for enumerating DPSCs requires new strategies. The purpose of this study was to verify a flowcytometry-based method for their evaluation based on cell surface markers.
Materials and Methods: Pulp tissues were digested using the routine procedure with collagenase and dispase tissue digestion. Half of the obtained single cells were collected and stained for the four colors STRO1-FITC, CD105-PE, CD271-APC and CD45-PerCP undergoing immediate analysis with flowcytometry. The other half of the collected cells cultured and after 3 passages, cells underwent flowcytometry for mentioned markers.
Results: Before culturing 29% of cells were CD45 positive indicating the presence of blood cells in the vessels of the samples. All the cells were negative for CD271. 25.8% of the cells were double positive for STRO1 and CD105 as the stem cell population. Cultured cells were fusiform fibroblast-like plastic adherent cells and were 35.9% positive for stem cell markers mentioned.
Conclusion: Also there is no significant difference between the 25.8 double positive percent before culturing with the 35.9 after culturing one; but this difference comes lower when the monocytes and lymphocyte become gated in the preculturing analysis. The other part of cells showing the fusiform appearance, are possibly fibroblasts isolated from pulps. Our study results shows that flowcytometric enumeration of DPSCs for comparative studies are possible but further studies for comparing this method with CFU-F assay is needed.
Keywords: Adult Stem Cells, Dental Pulp Stem Cell, Flowcytometric Analysis

Ps-10: Dental Pulp Stem Cells Do Not Show Some of Bone Marrow Derived Mesenchymal Stem Cells Surface Antigens

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Objective: Multipotent mesenchymal stromal cells (MSCs), previously known as "Mesenchymal stem cells", are a group of multipotent and clonogenic cells. These cells were primarily found among the bone marrow stroma. Dental pulp stem cells (DPSCs) in comparison with other sources of adult stem cells such as bone marrow have shown to have more replicative potentials while having the same differentiation capacity. For long, it has been considered that DPSCs are one type of tissue specific MSCs. Since they have the same differentiation potentials and also expressed the same cell surface antigen markers. The Goal of this study was to assess presence of newly find MSCs antigens on the DPSCs.
Materials and Methods: Pulp tissues were digested using the routine procedure with collagenase and dispase tissue digestion. The obtained single cells were collected and cultured. After 3 passages, cells underwent flowcytometry for CD73, CD34, CD45, CD14, CD90, CD44, CD166, HLA-DR, CD271, mesenchymal stem cell antigen1 (MSCA1).
Results: Dental pulp stem cells were fusiform fibroblast-like plastic adherent cells with similar features as bone marrow derived mesenchymal stem cells (BM-MSC) do. DPSCs are more than 90% positive for CD73, CD166, CD90, CD44 and negative for CD34, CD45, CD14. But in contrast with MSCs they were negative for CD271 and MSCA1.
Conclusion: It has been shown that MSCs are a heterogeneous population and in some reports they do express CD271 and MSCA1 les than 60% also these two markers have been used widely for purification and enrichment of MSCs. Regarding our data DPSCs are truly negative for these two antigens and positive selection of these markers are not useful for isolation of DPSCs.
Keywords: Adult Stem Cells, Dental Pulp Stem Cell, CD271, Mesenchymal Stem Cell antigen 1

Ps-11: Controlling Directional Migration of Neural Stem Cells Electrically

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Objective: Abstract embryonic stem cells from mouse strain (OCT4-GIP) was Cultured in medium (DMEM/F12) supplemented with leukaemia inhibiting factor (LIF) in Petri dishes treated with gelatin 0.1% and the cultures incubated in an incubator CO_2- temperature 36.9-36.7°C , and CO_2 partial pressure of 9.4 to 2.4%. Cultured cells responded to factors of culture and proliferated well, and dispersed new cultures new daily. Quoted cultures cellular processing of a special called electrotactic chamber contains the medium of culture and provided of circuit electrical particular, and provided chamber to control the PH, and also provided by installation of electrical control ensures the strong from 5.5V to 12V for the cells cultured. for these cultered cells the experiment revalated this cell (OCT4-GIP) move effectively and activity apparent when they exposed of the electric field in the electrotactic chamber, and that this movement much larger than the movement of the same cells cultured in the same conditions without electric field (control). this phenomenon is useful stimulating the migration of stem cells In vivo tissue towards the target of a terrorist (like wounds) to expedite and facilitate the healing, as well as directs the context of these cells to repair nerve tissue battered. Key words: Embryonic stem cells, Electric field, wound healing, Migration of embryonic stem cells.
Materials and Methods: Cells were seeded into electrotactic chamber coated with either gelatine (0.001%), or poly-ornithin-laminin. METAMORPH system was used for the electric fields (EF) direct current (dc) experiment. Electric power used from 500 volts onwards and current ranges between 5.5V to 12V. Two wavelengths were used: Green Fluorescence Protein (GFP), and BrightFiled. Each electric field and control experiments were carried out for a duration of 3-5 hours. Materials: -Medium with HEPES buffer(GIBCO, cat. no.15630-049) & LIF -Agar bridges, Agar (sigma, cat.no. A7002) -Glass slips for chambers -Coating gelatine and poly - orithin - laminin chambers -Steinbergs soluition -High vacuum grease Equipment: -Direct current power supply (CONSERT E143. and cables. -LAMBDA 10-2.Sutter instrument CO. Floresentes model ebq 100 ISOLATED -MetaMorph Microscope model ZEISS Axiovert S100TV -temperature T36.9°C -Computer model DELL -Voltage meter -Standard glass slides -Cover glass, size 22 × 22mm^2, 22 × 40mm^2 and 48 × 64mm^2 -Silver wire electrode (Advent,cat, no. AG549109) -Glass tubes (inner diameter approximately 7mm) -Glass-cuting diamond pen or glass saw -Tissue culture Petri dishes with 10 mm diameter electrical insulating compound silicone grease (DC4; Dow Corning) - Time-lapse imaging system, ideally with function of X/Y/Z multiple position recording and multiple wavelength recording, as well as a CO_2-supplied temperature control chamber incorpoated onto the microscope using the MetaMorph imaging system (Molecular Devices, UK). For result used program imageJ-mtrackJ and MetaMorph.
Results: Cells were cultured and seeded to passage 46 (P46). Cultured cells were very healthy and some were frozen and kept in liquid nitrogen tank. At one stage, cells were contaminated. Contaminated cells were sterilized with vortex and discarded. All culture media, chemicals and solutions for cell culture were changed to avoid any further contamination. Oct4 marker protein expresses pluripotency. Embryonic stem cells tagged with Oct4-GFP marker protein expresses pluripotency, once the

ES cells differentiate, Oct-GFP expression disappear and the cells lose pluripotency. Pluripotent embryonic stem cells were seeded in electrotactic chamber precoated with poly-ornithine/laminin for direct current electric field application. During direct current electric field application, 11V current was applied to electrotactic chamber containing a monolayer of stem cells/ neural progenitor cells and left for 5 to 7 hours. This results in migration of pluripotent embryonic stem cells at a short extent and also a significant migration of neural progenitor cells.
Conclusion: This experimental system may be used to simulate and study cellular and molecular responses to electric signals in the events of wound healing.
Keywords: Embryonic Stem Cells, Electric Field, Wound Healing, Migration of Embryonic Stem Cells

Ps-12: Effect of Serum Supplementing on Short Term Culture, Fate Determination and Gene Expression of Spermatogonial Stem Cells in The Goat

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Objective: To investigate the effect of serum supplementing on short term culture, fate determination and gene expression of spermatogonial stem cells (SSCs) in the goat.
Materials and Methods: Crude testicular cells were plated over Datura-Stramonium Agglutinin (DSA) for 1h, and non-adhering cells were cultured in presence of different serum concentrations (1, 5, 10, and 15%) for seven days in a highly enriched medium supplemented with B27-Vit A, GDNF, bFGF, EGF. The colonies developed in each groups were used for morphology assessment, immunocytochemistry (ICC) using different established SSC markers, and gene expression.
Results: The crude testicular cells extract contained subpopulation of cells that were positive for VASA (3.3%), OCT3/4 (1.0%), PLZF (0.04%), and Thy-1 (0.03%). Brief incubation with DSA resulted in three-fold increase in the number of cells that expressed germ cell marker. At all serum concentration, the SSCs maintained for one week. However, the expression of Thy-1, specific marker of undifferentiated spermatogonia, was significantly higher in colony cells developed in presence of 1% than the other groups.
Conclusion: Goat SSCs maintained in culture for one week in a highly enriched medium established in mice at concentrations of serum as low as 1%, while higher serum concentrations had detrimental effects on SSC culture/expansion.
Keywords: Goat, Spermatogonial Stem Cell, Serum

Ps-13: Differentiation of Inner Ear Hair Cells from Olfactory Stem Cells of Rat Olfactory Epithelium

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Objective: Many people over the world lose the hearing and turn deaf. One of the greatest challenges in the treatment of inner ear disorders is to find a cure for the hearing loss which is caused by the loss of inner ear cochlear hair cells or spiral ganglion neurons. So stem cell resources which are capable of differentiating into hair cells are necessary. Many studies aimed to differentiate stem cells to hair cell for example using embryonic stem cells and adult stem cells like neural stem cells. But using this type of stem cells have some problem for example embryonic stem cells have great potential to differentiate into hair cells, but they also have tumorigenic potential when transplanted or Neural stem cells do not differentiate to hair cells completely and not in a high amount. Rat olfactory mocusa contains stem cell population that can be cultured as a neurospheres and have multipotency to differentiate to different cell types. In this study we differentiated olfactory stem cells into functional inner ear hair cells.
Materials and Methods: Rat olfactory epitheliums were isolated and cultured in proliferation medium after enzymatic digestion and cell isolation. To induce inner ear hair cells differentiation, the cells were cultured in differentiation medium after fifth passage. For characterization of the hair cells RT-PCR for three specific gene was done, include : Brn 3.1, Math 1, Myosin IIV, and immunocytochemistry assays were used for some specific markers such as: P27(DCS-72,SPM 348), Myosin VIIa (C-5,A-16), MATH-1(Y-23, N-19). Morphological properties were also considered.
Results: Hair cells differentiation was confirmed by expression of multiple genes related to this type of cells by RT-PCR and also differentiation was assessed by immunoflorescence staining using specific antibodies. Hair cells were also confirmed by their morphologic characterization.
Conclusion: Our results demonstrated that olfactory stem cells obtained from olfactory epithelium of rat has a differentiation potential to produce hair cells of inner ear *in vitro* therefore it can provide a potential source of autologous cells for transplantation.
Keywords: Olfactory Stem Cells, Olfactory Epitheliums, Hair Cells, Hearing Loss, Differentiation

Ps-14: Differentiation of Mesenchymal Stem Cells Derived from Bone Marrow to Lens Fiber Cells

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Objective: Stem cells are defined as a population of primitive cells with capability of self-renewal and differentiation into multiple cell lineages. Hematopoietic stem cells and mesenchymal stem cells (MSCs) are two main stem cell populations housed within adult bone marrow.Vitreous body including FGFs such as FGF-1 and FGF-2 in the processes involved in lens induction, differentiation, and elongation, structural specialization, and onset of specialized crystallin gene expression. Crystallins, divided into two major families α and βγ are the major proteins of the lens. The aim of this study is conducted to determine the effects of vitreous humor on the mesenchymal stem cells (MSCs) derived from bone marrow to lens fiber like cells.
Materials and Methods: Bone marrow was collected by flushing femurs and tibias in NMRI mice and then plated in 25-cm^2 plastic culture flasks. Owing to their plastic adherent property, MSCs attach to the bottom of flask but hematopoietic stem cells were removed by changing the culture medium. Immunocytochemistery have achieved to confirm stemnese cells using Oct4 antibody Then, MSCs were treated with other supplemented and bovine vitreous body for induction, the supplemented medium was changed every two days. The expression of differentiation marker, αA- crystalline, was detected by Immunocytochemistery.
Results: During the primary culture, the cell population was heterogeneous where varying morphologies Such as flat, spindle-shaped, and polygonal cells were observed. In the subsequent passages, the number of the spindle-shaped cells appeared to increase, so that in assage 3 the majority of the cells seemed morphologically to be spindle-shaped. Morphological studies showed that most cells in experimental group cells were locally longer and more aligned in parallel compared to control group cells. Also, the fiber like cells had large nuclei with multiple nucleoli. Immunocytochemistery studies confirmed the stemness cells and lens fiber cells.
Conclusion: According to the findings of this study, it can be concluded that MSCs derived from mouse bone marrow differentiate into lens fiber cells by treating them with vitreous humor.
Keywords: Mesenchymal Stem Cells, Vitreous Body, Lens Fiber Cells, Crystallin Bone Marrow

Ps-15: Neuronal Differentiation of GFP Expressing P19 Embryonal Carcinoma Stem Cells

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Objective: P19 cells are a line of pluripotent embryonal carcinoma stem cells able to grow continuously in serum-supplemented media and can be induced to differentiate along either mesodermal or ectodermal lineage. The differentiation of these cells can be controlled by nontoxic drugs. When treated with deprenyl, P19 cultures differentiate into cell types similar to those derived from neuroectoderm. The antiparkinsonian effect of deprenyl was reported by several investigators. On the other hand, there is a growing interest in the potential use of stem cell therapy in Parkinson's disease. One of the major advantages to using P19 cells in the study of neuronal function and development is that these cells are amenable to genetic manipulation. Foreign DNA can be introduced into these cells using a standard method involving calcium phosphate precipitation. In this investigation, deprenyl was used to induce neuronal differentiation in undifferentiated pluripotent P19 embryonal carcinoma cells. We used calcium phosphate precipitation method to transfect these cells with pML8 plasmid, a vector encoding eGFP and puromycin resistance gene under the control of the murine Pgk-1 promoter.
Materials and Methods: The cells were cultured using α-MEM medium that supplemented with 15% fetal bovine serum (FBS). In the treatment group, deprenyl was used to induce embryoid body (EB) differentiation to neuronal lineage. Initially, the viability test was used to select the range of nontoxic doses before transfection. The optimal inducing dose was obtained using different concentrations of deprenyl (10-6–10-11 M). The viability of untransfected P19 cells during the experiment was determined by the trypan blue dye exclusion method. The peak response was at 10-8 M, which was used for further investigation. Morphologic and immunofluorescence techniques were used to evaluate the differentiation of the P19 cells, Cresyl violet for the morphologic study, anti-synaptophysin and anti-betatubulin III antibodies for characterizing the neuronal phenotype of the cells.
Results: The results show that deprenyl can induce neuronal phenotype associated with neuronal marker expression in successfully GFP-transfected P19 carcinoma cells. It induced the differentiation of the cells into neuron-like cells in a concentration-dependent manner.
Conclusion: This study suggests the potential use of combined deprenyl and stem cell therapy to improve deficits in neurodegenerative diseases.
Keywords: Embryonic Carcinoma Stem Cell, GFP Transfection, Neuronal Differentiation, Synaptophysin

Ps-16: Generation of Transgenic Murine iPS Cells by a Single Polycistronic Lentiviral Vector

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Objective: Induced pluripotent stem cells or iPS cells are a type of pluripotent stem cell artificially derived from a non-pluripotent cell, like fibroblasts, by inducing the expression of pluripotency-related genes and, thus, the transcriptional network of pluripotency.iPS Cells are similar to the natural pluripotent stem cells derived from the blastocyst's inner cell mass in many aspects, such as expression of key stem cell markers like Oct4, Sox2 and Nanog. As various disease-specific mouse strains are well-studied, murine iPS cells are good models for basic research on developmental disorders or applying high-throughput screens that need vast amounts of the respective diseased cell type.
Materials and Methods: In our study, we isolated fibroblasts from tail tips of mice that harbored an alveolar cell-specific reporter transgene. After isolation and initial propagation, the fibroblasts were transduced with a lentiviral reprogramming vector (MOI= 20) in the presence of protamine sulphate and valproic acid. This reprogramming vector contained a polycistronic reprogramming cassette consisting of human codon-optimized cDNAs of Oct4, Sox2 and Klf4 as well as of an IRES-coupled tdTomato reporter sequence. After characteristic changes of morphology towards iPS-like colonies the media was changed to 1:1 fibroblast/ embryonic stem cell medium, then the cells were transferred to mitotically inactive C3H feeder cells and cultured with plain embryonic stem cell medium.
Results: The characterization of iPS cells demonstrated alkaline phosphatase activity and a strong expression of endogenous pluripotency factors by real time PCR. Furthermore, immunocytochemistry confirmed the pluripotent phenotype by strong signals for Nanog, which is independent from the applied reprogramming factors
Conclusion: Our experiments showed that obtaining murine iPS cells from transgenic mouse fibroblasts is feasible through a single polycistronic plasmid coding for Oct4, Sox2 and Klf4.
Keywords: Murine Induced Pluripotent Stem Cells (iPSC), Lentiviral Vectors, Reprogramming

Ps-17: Ayurvedic Rasayana Herbs As a Sex Health Tonic

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Objective: Rasayana are a group of herbal drug preparations widely used in Ayurveda to improve the general health of the body. Rasayana therapy has capability of nourishing and rejuvenating drugs with multiple applications for longevity, memory enhancement, immunomodulation and adaptogenic. Vajikaran is one of the eight branches that deal with improving male sexual potency and thereby ensuring a supraja, or better progeny. The main aim of Vajikaran besides achieving successful copulation for healthy reproduction, with sexual pleasure is an additional benefit. Many researchers have supposed neuroendocrine immune axis theory to explain Rasayana action. For proved this theory by selecting the plants of rasayan category. The plant Curculigo orchioides, Astercantha longifolia, Mucuna pruriens, Pueraria tuberosa, Bryonia lacinosa, Chlorophytum borivilianum and Anacyclus pyrethrum are well known vaajikaran rasayan herbs.
Materials and Methods: The study was therefore performed to effect of these plants on reproductive parameters. Following parameters were evaluated the effect of extract on body and organ weights, mounting behavior, orientation behavior, change in histoarchitecture of testis and epididymis, fructose level in seminal vesicles and hormonal level was studied. All these herbs also screened for imunomodulatory and antioxidant potential.
Results: Administration of ethanolic extract had pronounced anabolic and spermatogenic effect in treated animals as evidenced by weight gains in the body and reproductive organs. Increase in spermatogenesis was shown in all treated group. The level of follicular stimulating hormone, leutinizing hormone and testosterone level is significantly increased in extract treated group and fructose content in seminal vesicles was significantly increases in treated groups. All herbs showed strong antioxidant and immunomodalatory activity.
Conclusion: Thus it was concluded that drug was justifying the use in the traditional system of medicine as a vaajikaran rasayana and used as health tonic
Keywords: Aphrodisiac, Herbal, Ayurveda, Rasayana, Spermetogenesis

Ps-18: Dexamethasone Reduces Doxorubicin-Induced Oxidative Stress and Caspase 3 Activity in mESC-Derived Pure Cardiomyocytes

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Objective: Cardiac toxicity remains a serious yet unsolved complication of doxorubicin. This study was designed to examine whether dexamethasone, a anti-inflammatory and immunosuppressive action of synthetic glucocorticoid with potential cytoprotective properties, could ameliorate this complication of doxorubicin. The exact mechanism for doxorubicin cardiomyopathy is not clear, but reactive oxygen species generation and myocardial cell apoptosis may contribute. It has further been reported that DOX induces apoptosis in cardiomyocytes in specific conditions. Moreover, it has been revealed that mitochondrial dysfunction is a critical factor inducing apoptosis. Therefore, we hypothesized that DOX induces apoptosis through caspase activation via the mitochondrial pathway in cardiomyocytes and plays an important role in DOX-induced cardiomyopathy. we tested this hypothesis using genetically engineered mouse embryonic stem cell - derived cardiomyocytes.
Materials and Methods: In this research, Mouse transgenic ESCs line RB1α-MHC differentiated cardiomyocytes (CMs) were purified under puromycin (pur) treatment. The percentage of purified CMs were assessed by flow cytometry. Beating cardiomyocytes were treated with different concentrations of doxorubicin (DOX), dexamethasone (DEX), mifepristone (MIF) or combinations of them. Percentage of beating cardiomyocytes, MTS assay for cell survival and caspase 3 activity using an assay kit were evaluated in each group (DOX, DEX (24 hours) + DOX (24 hours), MIF (30 minutes) / MIF+DEX (24 hours) + DOX (24 hours) and CON groups.
Results: Highly purified α-MHC+ cardiomyocytes have been generated through 7 day puromycin treatment of transgenic RB1containing green fluorescent protein (GFP) reporter and puromycin-resistant gene under the α-MHC promoter.During this treatment in contrast to non puromycin resistant cells, puromycin resistant 15-day-old EGFP-expressing α-MHC+ cardiomyocytes were gradually enriched. Not only doxorubicin cardiotoxicity on mouse transgenic embryonic stem cell-derived beating cardiomyocytes were ameliorated by treatment with dexamethasone (DEX) when DEX administrated 24 hours before DOX, but also cell survival increased. When cardiomyocytes were incubated with Dox for 24 hours, an elevated activity of caspase-3 was observed with Dox at 5μM or above. Caspase-3 activity assay indicated that 5μM was an appropriate dose for inducing apoptosis. Interestingly, caspase-3 activity was significantly decreased in DEX-pretreated groups.
Conclusion: This study indicated that CT inhibits apoptotic-like cell death induced by Dox in primary cultured cardiomyocytes. Also, DEX pretreatment causes increment in cell surveillance in and decrement in caspase-3 activity in DOX-treated cardiomyocytes. These data open a window for effective prevention of this devastating cardiac complication by adjuvant administration of the glucocorticoids, which might provide a solution to this otherwise severe and potentially fatal complication of doxorubicin and may provide us with a clinical suggestion which proposes that the beneficial effect of DEX is obtained when DEX was added before DOX administration.
Keywords: Engineered Mouse Embryonic Stem Cell, Cardiomyocytes, Doxorubicin, Dexamethasone, Mifepristone, Viability, Caspase 3 Activity, Cardiotoxicity

Ps-19: Construction and Characterization of Specific Non-Viral Vector for *Ex vivo* Expression of Human β-Globin Gene Using Induced Pluripotent Stem (iPS) Cells

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Objective: The invention of induced pluripotent stem (iPS) cells enables for the first time the derivation of enough numbers of patient-specific stem cells and holds promise for cell-based therapies. Recent studies have revealed the potential of iPS cell generation combined with gene therapy for treatment of different disease in human. In this study we designed and constructed a nonviral plasmid vector as a gene transfer vehicle. This vector is tissue-specific and its integration in the genome is site specific. We expect to have persistent and acceptable level of β-globin expression as transgene per genomic copy from the vector. This vector will be used for genetic manipulation of iPS cells derived from patients with β-thalassemia major or sickle cell anemia with potential for clinical application.
Materials and Methods: In the first step, an appropriate nonviral plasmid vector was designed and constructed by genetic engineering approaches. This vector comprises two main parts, a bacterial backbone for efficient amplification in *E. coli* cells and one expression cassette containing β-globin gene enhancers, promoter and β-globin gene sequence along with its 3′ & 5′ UTRs and one eukaryotic antibiotic resistance gene against puromycin. By adding a specific phage integrase site in

the structure of this vector, integration occurs in specific sites in the genome (Site Specific Recombination; SSR). Also by designing of two loxP sites flanking of bacterial backbone and puromycin ORF it is possible to delete these sequences by Cre recombinase after integration of vector in the genome of target cells. These characteristics reduce possible host immune responses to the vector and potential genotoxicity. The structure of the vector was confirmed through several steps of digestion experiments and sequence analysis. Functional assessment of plasmid vector, which is named pHBB, was achieved by transient transfection in to hematopoietic cell lines, which are β-globin promoter specific and able to express β-globin gene.
Results: The recombinant expression vector containing β-globin gene was successfully constructed and confirmed. To study of its activity, the vector lipofected in to suitable hematopoietic cell lines transiently and the expression of the gene under control of β-globin promoter was detected.
Conclusion: This nonviral plasmid vector is applicable to correct the defects of β-globin gene by adding a functional copy of β-globin gene in stem cells derived from the patients who have the β-thalassemia major or sickle cell anemia (gene addition method) using site specific recombination. The production about 10% or more hemoglobin in RBCs of these patients compare to normal people can help to treat these disease.
Keywords: β-Globin, Nonviral Vector, Site Specific Recombination, iPS Cell

Ps-20: Amniotic Fluid Mesenchymal Stem Cells and Curing Eye Disease

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Objective: The developing fetus is surrounded by a protective layer of liquid, amniotic fluid, that provides mechanical protection as well as nutrient required for fetal growth and well-being and contains cells derived from embryonic and extra embryonic tissues. One of the cell types that are also believed to be part of the cellular content of amniotic fluid are mesenchymal stem cells (MSCs). These cells possess high proliferation, are OCT4 positive and are able to differentiate into cell types of three germ layers, like adipogenic, osteogenic, myogenic and endothelial cell types, neurogenic cell types and hepatic cell types. So that MSCs with high potential of differentiation can be used to cure different diseases. The aim of this study is to isolate MSCs from amniotic fluid and differentiate them into lens fiber cells.
Materials and Methods: MSCs were isolated from pregnant NMRI mice in the second week of pregnancy. The adherent characteristic to the plastic plates was used to isolate cells in the first step. Vitreous body was used as treatment medium. After three passages, cells were cultured with vitreous body, DMEM, FBS and antibiotics for 14 days. MSCs and treated cells were identified by morphological and immunocytochemical studies.
Results: The isolated cells from AF showed fibroblast-like morphology. The expression of OCT4 that is a special factor of stem cells in MSCs was demonstrated with immunocytochemical studies. Immunocytochemicall studies showed that treated cells expressed crystallins, which are special factors of lens fiber cells.
Conclusion: Amniotic fluid MSCs are a new source of cell types to cure eye diseases, like Cataract and under certain conditions these cells can be differentiated into lens fiber cells *in vitro*.
Keywords: Amniotic Fluid, Mesenchymal Stem Cells, Vitreous Body

Ps-21: PPARγ effects on Mouse Embryonic Stem Cells Proliferation

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Objective: Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor super-family of ligand-activated transcription factors and are comprised of three isoforms (α, β and γ) which are encoded by three distinct genes. PPARγ is mostly expressed in adipose tissue and the immune system. PPARγ consists of two variants, γ1 and γ2. PPARγ1 is expressed in relatively low abundance within many tissues. PPARγ has many effects on cell activities like cell differentiation, adipogenesis, inflammation and apoptosis. Thus the aim of this study is to assess the effects of PPARγ on state of proliferation of mouse embryonic stem cells.
Materials and Methods: Different concentrations of PPARγ agonist and antagonist were implemented for mouse embryonic stem cells culture. Total cell were harvested after treatment and Real time PCR analyses performed along with flowcytometry.
Results: Our data indicated that PPARγ acts through its agonists (Rosiglitazone and Ciglitazone) increased mESC proliferation in the presence of LIF whereas PPARγ antagonist (GW9662) reversed the effects caused by the PPARγ agonists. However, treatment

of undifferentiated mESCs with PPARγ agonists had no influence on future differentiation. These results suggest a possible role of PPARγ on mESC self-renewal of LIF.
Conclusion: PPARγ has a critical role on embryonic stem cell proliferation.
Keywords: PPARγ, mESC, Proliferation, LIF

Ps-22: Compression of RUNX2 Quantitative Expression in Mesenchymal Stem Cells Differentiated with Osteoblastic Differentiation Medium and Zoledronic Acid

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Objective: RUNX2 is the most specific transcription factor in osteoblastic differentiation of MSCs. Zoledronic acid is a nitrogen-containing bisphosphonate. It is used to prevent skeletal fractures in patients with cancers, as well as for treating osteoporosis. Mechanism of zoledronic acid on osteoblastic differentiation of mesenchymal stem cells (MSCs) has not fully understood. In this research, RUNX2 expression were quantified in MSCs differentiated by osteogenic differentiation medium (ODM) and zoledronic acid (ZA).
Materials and Methods: Prepared human mesenchymal stem cells were treated by osteoblastic differentiation medium containing dexamethazone, β-glycerol phosphate and ascorbate-2-phosphate for 21 days. Also, mesenchymal stem cells were treated By zoledronic acid in 5μM concentration for 3 hours. Then, they cultured in osteoblastic differentiation medium for 21 days. RNA extraction was carried out in both undifferentiated mesenchymal stem cells and osteoblastic differentiated cells in days 7, 14 and 21. Osteoblastic differentiation was confirmed by alizarin red staining. RUNX2 expression was quantified by quantitative Real Time-PCR.
Results: Gene expression of RUNX2 in first, second and third weeks of osteogenic differentiation by osteoblastic differentiation medium compared to undifferentiated mesenchymal stem cells showed 1.7-fold, 3.5-fold and 3.4-fold increase in expression, respectively.RUNX2 expression shoed 2.9-fold, 3.2-fold and 3.3-fold increase in the same intervals of osteogenic differentiation by zoledronic acid.
Conclusion: RUNX2 expression increased in osteoblastic differentiation by both osteoblastic differentiation medium and zoledronic acid. Increase of RUNX2 expression in week 1 by zoledronic acid showed that zoledronic acid accelerate osteoblastic differentiation.
Keywords: Mesenchymal Stem Cells, Osteoblastic Differentiation Medium, Zoledronic Acid, RUNX2

Ps-23: The Generation of Living Skin Containing Biocompatible Scaffold and Stem Cells and Its Evaluation in Regeneration of Experimental Burned Animal

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Objective: Burning is the first and most common injury of skin. Although burn patients are faced with many problems but the most important issue for these patients is burn repair and scar. Stem cell is a non specialized cell that has the ability to produce identical cells and also have the ability to differentiate into the many specialized cells like skin keratinocytes. The whole purpose of this study is culture the differentiated stem cells cultured on a biodegradable scaffold to produce a three-dimensional living skin tissue.
Materials and Methods: The bone marrow (from Tibia bone) and fat tissue (from Scrotum) of rat were allowed to extract mesenchymal stem cells.Extracted MSCs were identified after separation and proliferation by examining specific surface markers such as CD44, CD45, CD73, CD90 by flowcytometry.The capability of MSCs to differentiate along adipogenic and osteogenic lineages was confirmed at passage 3. For differentiation of MSCs into skin keratinocyte cells, the differentiation medium including DMEM/ Ham`s F12 (3:1) + 10 % FBS + 1% Pen/Strep + 5 μg/ml Insulin + 0.5 μg/ml Hydrocortisone + 1.5 mM $Cacl_2$ + 10 ng/ml EGF + 10 ng/ml KGF was used . Differentiation of stem cells into keratinocytes were checked by using immunocytochemistry (ICC) method (Pan-keratin and Involucrin anti bodies) and reverse transcriptase polymerase chain reaction (RT-PCR) with cytokeratin 18 and involucrin genes. The prepared cells need an appropriate support with particular characteristics, including mechanical properties and good biocompatibility to resemble more likely to normal skin. For this reason, a scaffold should be provided which allowed holding and binding cells to its surface, finding not to be toxic, does not prevent cell growth and proliferation and have appropriate mechanical and physical proprieties.After consideration of various materials and polymers, a scaffold composed of Chitosan and collagen of 10:90 ratios was used. Then differentiated cells with biocompatible Chitosan-Collagen scaffold were autografted to the burned rats.
Results: Results of ICC and RT-PCR tests confirmed

the successful chemical differentiation. After about 2 weeks the differences between grafted scar and control one was significant. Also after about 20 days, grafted scars healed completely but full recovery weren't observed in control samples.
Conclusion: In these experiments we showed that differentiated mesemchymal stem cells into skin keratinocyte with a biocompatible scaffold can improve the wound healing. So in future it can be used in humans as a good way to repair scars, specially burn scars.
Keywords: Mesenchymal Stem Cell, Differentiation, Keratinocyte, Scaffold

Ps-24: Autologous Human Plasma As a Substitute to Fetal Bovine Serum for Expansion of Bone Marrow Mesenchymal Stromal Cells

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Objective: Bone marrow mesenchymal stromal cells (BMSC) are promising candidates for cell-based therapies. However, most isolation and expansion protocols for clinical-scale production of MSCs use fetal calf serum (FCS) as a supplement. To find a suitable FCS substitute and less contamination probability, we investigated the effects of autologous human serum and plasma components on bone marrow MSCs expansion and morphology.
Materials and Methods: Bone marrow stromal cells were seeded on 100 mm plates and T75 flasks with three different media A, B, C, containing 10% selected FCS, 10% autologous serum (AS) or with blood components, respectively. After primary culture, Cells was passaged to fresh or half MSC-conditioned media.
Results: First, in compare to conventional Ficoll density gradient for blood depletion, non-isolation of bone marrow stromal cells from blood components, media culture C, exhibited feasible and intensive expansion of MSC. We observed that human autologous plasma and serum demonstrated a significantly higher proliferative effect on MSCs, with C > B > A media. Moreover, MSC-conditioned medium, providing autocrine growth factors appears to enhance and retain proliferative capacity of MSC throughout long-term culture.
Conclusion: Human growth factor resources are better supplements regarding reduced-potential infectious threats, providing a higher proliferation rate and retaining differentiation capacity, whereas FBS impose a potential risk for infections as well as immunological reactions. Thus human blood products offer certain advantages as potential substitutes for clinical cell therapy.
Keywords: Mesenchymal Stromal Cells, Fetal Bovine Serum, Autologous Human Serum, Autologous Plasma Components, MSC- Conditioned Medium

Ps-25: Selecting Optimal Cultivation of Human Bone Marrow Mesenchymal Stem Cells for Clinical Cell Therapy

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Objective: Clinical application of human mesenchymal stromal cells (hMSCs) and successful stem cell therapy requires their expansion to be safe and rapid. We aimed to evaluate the alternatives of isolation and cultivation methods of hMSC to develop a protocol which would shorten the expansion time and avoid multiple passaging to reduce contamination risks.
Materials and Methods: First, we have compared the expansion of the Ficoll gradient - isolated and non-isolated cell suspensions in alpha-MEM and DMEM medium, separately. Then, co-culture and MSCs-conditioned medium was evaluated for expansion and proliferation capacity of the cells.
Results: When the Ficoll gradient density isolation was used and non-adherent cells were discarded, both media were comparable whereas, significantly more hMSCs were obtained from non-isolated bone marrow cultures in DMEM medium. Furthermore, the numbers of hMSCs were significantly higher when non-adherent cells or supernatants were not discarded. However, flask-culture system seemed more favorable and led to increase of hMSCs after two weeks of cultivation compared to 100 mm plate cultures.
Conclusion: Our experiment thus, admits previous observations and leads to a protocol for more effective hMSC production which can be used in cell therapy.
Keywords: Isolation, Expansion, Conditioned- Medium, Mesenchymal Stromal Cells

Ps-26: PPARγ Influences on Neural Precursor Cells Formation and Neural Differentiation of Mouse Embryonic Stem Cells

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Objective: Peroxisome proliferator activated receptors (PPARs), consisting three major isoforms termed α, β/δ and γ, are ligand-dependent transcription factors which are classified as one of the members of nuclear hormone receptors super family. Among them, PPARγ is involved in different metabolic activities including lipid metabolism, anti-inflammatory responses and regulation of tissue homeostasis, insulin sensitivity, development and differentiation. To address the importance of PPARγ on neural differentiation of mouse embryonic stem cells, the present study was designed and carried out during two different stages in process of neurogenesis by application of PPARγ selective agonist and antagonist.
Materials and Methods: Royan B1 cell lines derived from the C57BL/6 strain were treated with either Rosiglitazone or GW9662, during or post neural precursor cell formation. Different approaches as Real Time PCR, immunostaining were implemented to chase the fate of neural cells during differentiation.
Results: Data indicated that a decrease in the expression level of mature astrocyte marker post neural precursor cell formation treatment by inactivation of PPARγ.
Conclusion: PPARγ is able to modulate the rate of neural differentiation of mouse embryonic stem cells.
Keywords: Neural Differentiation, PPARγ, Mouse Embryonic Stem Cells

Ps-27: Simple and Efficient Method for Cancer Stem Cells Isolation from Human Breast Cancer Cell Line MDA-MB231

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Objective: Since cancer stem cells (CSCs) hypothesis has emerged, cancer has been considered as a stem cell disease. Due to some problems in isolation of CSCs throughout methods like FACS and MACS, establishment culture combined with anti cancer drugs has recently been used to do so. In current study, vincristine as an antimitotic drug has been used in order to isolation and purification of CSCs from human breast cancer cell line MDA-MB231.
Materials and Methods: There were two sets of experiments: At first, Determination of optimal dose of vincristine in order to isolate cancer stem cells from human breast cancer cell line MDA-MB231. Mentioned, conducted by cells treatment with 2, 4, 6 and 8 ng/ml of vincristine (72 hours) followed by MTT assay and trypan blue dye exclusion. Then, identification, purification and characterization of isolated cells which was performed by RT-PCR and immunofluorescence staining upon exposure to vincristine, culture of cells in non adherent culture condition in CSC medium and formation of mamosphere forming unit (MFU); respectively.
Results : Both MTT and trypan blue dye exclusion assay demonstrated significant decrease in viability of cells treated with high doses of vincristine (6 and 8 ng/ml) in comparison with low ones (2 and 4 ng/ml) ($p<0.05$). So, the later (4 ng/ml) was selected as an optimal dose of vincristine in order to combine with establishment culture condition. RT-PCR showed isolated cells upon exposure to vincristine expressed Oct3/4, Nanog, Sox2 and nocleostemin. Also, immunofluorescence staining demonstrated that isolated cells were CD44 positive. Furthermore, results of MFU assay displayed remarkable increase in percentage of MFU among cells pretreated with vincristine (3.3%) in comparison with those were not treated (0.4%) ($p<0.05$).
Conclusion: Non adherent culture condition in CSC medium (establishment culture) combined with vincristine can be considered as an appropriate method for cancer stem cells isolation from human breast cancer cell line MDA-MB231.
Keywords: Breast Cancer, Cancer Stem Cell, Isolation, Vincristine

Ps-28: Effects of PPARγ Agonist and Antagonist Treatment on Cardiac Differentiation of Mouse Embryonic Stem Cells

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Objective: Mouse embryonic stem ells with pluripotent characteristics, are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. One major group of nuclear receptor proteins in stem cells are peroxisome proliferator-activated receptors (PPARs) play essential roles in the regulation of cellular differentiation, development, and metabolism. This receptor family consists of three major isoforms termed α, β/δ and γ. Our previous studies have shown that PPARγ ligand involved in neural differentiation of mouse embryonic stem cells. In this study, the effects of PPARγ agonist and antagonist were investigated on cardiac differentiation

of mouse embryonic stem cells.
Materials and Methods: To formation of cardiac precursor cells, embryoid bodies were derived from mouse embryonic stem cells by 2days culture in hanging drops followed by 5 days in suspensions in KDMEM with 15% FCS. To differentiate and formation of cardiac cells, the embryo bodies were plated in KDMEM. To testify the involvement of PPARγ agonist and antagonistincardiac differentiation, agonist (Rosiglitazone) and antagonist (GW9662) of PPARγ were added during 4 days of suspension culture or the 7days of post plating.Finally expression of cardiac markers (α-cardiac actin, α-MHC) and smooth muscle markers (SMAα, SM22α) was evaluated in cardiac cells in both of treated and untreated cells.
Results: Data indicated the expression of α-cardiac actin and α-MHC as cardiac markers decreased significantlyin presence of PPARγ antagonist(GW9662) during the suspension culture treatment while the expression of smooth muscle markers were not influenced by this antagonist at that stage. Interestingly nor agonist and neither antagonist treatment effectively influenced the rate of cardiac differentiation at post plating stage.
Conclusion: Our data indicated that PPARγ activity may influence cardiogenesis commitment of embryonic stem cells during suspension culture of embryoid bodies.
Keywords: Peroxisome Proliferator-Activated Receptorγ, Mouse Embryonic Stem Cells, Cardiogenesis, Differentiation

Ps-29: Comparison of Gene Expression of Dopaminergic Neurons in Mesenchymal Stem Cells before and after Induction by Deprenyl

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Objective: The purpose of this study is the medicine neuroprotective effect of Deprenyl on dopaminergic gene expression sonic hedgehog (Shh), patched (Ptch), Nurr-1 and tyrosine hydroxylase (Th) in mesenchymal stem cells (MSCs) . If these cells express factors mentioned above, can be used as replacement cells for treatment of Parkinson's disease.
Materials and Methods: MSCs were isolated from the femor and tibia bone marrow of adult rats and cultured. After the cell culture and preparing subculture of the fourth or fifth passage were used to continue working. The cells were placed in two groups:
1. Cells grown in the α-MEM medium (Gibco) supplemented with FBS serum 10%.
2. Cells cultured in α-MEM medium with Deprenyl 10-8 M (0.00000001M) concentration To determine the purity the cells were stained with alkaline phosphatase and fibronectin antibody immunocytochemistry. Dopaminergic gene expression was evaluated with RT-PCR method.
Results: The results showed that 97% of cells had positive response to alkaline phosphatase and anti fibronectine staining. Based on RT-PCR results indicated that dopaminergic genes were expressed in both groups, but in terms of quality the gene expression in Deprenyl-induced group was higher than uninduced MSCs .
Conclusion: Mesenchymal stem cells with the expression of dopaminergic genes TH, Ptch, Nuur-1, Shh can play an important role in the treatment of neurodegenerative diseases and also medium containing low dose of Deprenyl could effect on increasing the expression of these genes.
Keywords: Dopaminergic Neuron, Mesenchymal Stem Cell, Deprenyl

Ps-30: Effect of Different Concentration of Staurosporine on Neurite Outgrowth in Mouse Bone Marrow Mesenchymal Stem Cells

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Objective: Bone marrow mesenchymal stem cells (BMSCs) can be differentiated into neuronal and glial-like cell types under appropriate experimental conditions. Staurosporine (STS), is a well known protein kinase inhibitor and apoptosis inducer that indicated has strong effect on neurite outgrowth in several types of cells. This study was undertaken to evaluate the effects of different concentration of staurosporine on cell viability and neurite outgrowth in cultured mouse BMSCs.
Materials and Methods: mBMSCs were isolated from the femur and tibia of NMRI mice and cultured in DMEM supplemented with 10% FBS at 37°C in humid air with 5% CO_2. Differentiation assay were done to affirmation multipotency of isolated cells by histological staining using sudan III for adipogenic and alizarin red for osteogenic differentiation. CFU-F assay was used to indicate mesenchymal stem cells. Cells were treated with different concentration (0, 50, 100, 200 and 300 nM) of staurosporine, treatments I, II, III, IV and V; respectively. The viability of cells was assessed by neutral red uptake assay in different period times (6, 12, and 24 hours). Total neurite length was estimated by measurement of the length of the longest neurite from the cell body to growth cone after 6, 12 and 24 hours.
Results: Typical neuron-like cells were identified after 6h and cells had typical morphological features of neurons. The viability assay showed that the viability of cells in treatments I, II and III is higher than the other treatments ($p<0.05$). Total neurite length measurement showed that neurite outgrowth was increased in all treatments from 6 hours to 24 hours. After 24 hours longest neurite was observed in treatment II and shortest

neurites were related to treatment V ($p<0.05$).
Conclusion: Our result suggests that staurosporine can be induces neurite outgrowth in a dose dependent manner.
Keywords: Marrow Mesenchymal Stem Cells, Staurosporine, Neurite Outgrowth, Mouse

Ps-31: PEP Gene Knock Down in Mouse Embryonic Stem Cells Decreased Significant Rate of Neural Differentiation

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Objective: Peroxisomal protein (PEP) is one of the peroxisomal matrix protein comprises 209 aminoacid residues. Our recent data indicated that PEP expression level was increased upon retinoic acid treatment duringneural differentiation of mouse embryonic stem cells. In order to further look in to the PEP function during neural differentiation, knocking down of PEP expression performed by means of RNAi approach .
Materials and Methods: Several siRNAs was designed using different bioinformatics softwares to silence PEP gene expression. The most appropriated siRNAs with length of 19 nucleotides, were selected along with a negative control siRNA(scramble). oligonucleotides were inserted into pSingle TTS-ShRNA inducible vector at HindIII and XhoI sites. Recombinant vectors were transfected into the mouse embryonic stem cells. Stably transformed colony cells expressing PEP shRNA were selected after antibiotics treatment. Doxycycline was used to induce the production of PEP shRNA. Cells were allowed to differentiate. Real Time PCR used for analysis gene expression based on $\Delta\Delta$ ct methods.
Results: Real time quatitative RT-PCR revealed that PEP knock down significantly decreased the expression level of neural markers (MapII, TujI) during neural differentiation.
Conclusion: These data confirmed a possible role of PEP during neural differentiation.
Keywords: PEP, Neural Differentiation, RNAi, Embryonic Stem Cell

Ps-32: Determination of Stem Cell Localization in Term Human Placenta by Immunohistochemical Study

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Objective: Stem cells are found in all multi cellular organisms. They are characterized by the ability to renew themselves mitotic cell division and differentiate into a diverse range of specialized cells. Recent reports indicate that the human placenta contains various types of stem cells, which are the mesenchymal, hematopoietic, trophoblastic and multipotent/pluripotent stem cells. This study was done with the hope that stem cells could be derived from different parts of the placenta, a tissue that is often by discarded. OCT-4 is critically involved in the self-renewal of undifferentiated embryonic stem cells. CD90 and CD200 can be used as markers for a variety of stem cells. The HOPE technique provides an excellent preservation of protein antigenicity and presents well, the morphological details in paraffin embedded placenta tissues. In this current study, we investigated the expressions of OCT-4, CD90 and CD200 in five different parts of placenta.
Materials and Methods: We studied the expression of CD90, CD200, and OCT-4 in 5 placenta tissues by fixated in HOPE fixative followed by immunohistochemical staining. Samples were taken from five different areas (amnion, chorion, chorionic villi, decidua basalis ,cord matrix) of each placenta and were incubated by different antibodies (CD90, CD200, and OCT-4). The number of positive cells in 100 cells were counted in the five areas and compared. The area in the placenta where stem cells were best localized was identified.
Results: In this study of five placentas we were successful in finding stem cells in different parts of placenta. The number of embryonic stem cells in different areas of the placenta exceeded the adult stem cells the richest area being the amnion and the chorionic villi. Higher expression OCT-4 can give us this idea; placenta can be a good source of pluripotent stem cells.
Conclusion: The number of embryonic stem cells in different areas of placenta are more than adult stem cells. The amnion and the chorionic villi are the richest sources of stem cells. The highest expression of OCT-4 has proven that the placenta is a good source of pluripotent stem cells. Placenta provides an easily accessible and cheap source of stem cells, and its potential needs to be explored to the fullest with further researches.
Keywords: Embryonic Stem Cell, Placenta, Immunohistochemical Staining, HOPE Fixative

Ps-33: Uniaxial Cyclic Loading Effects on the Differentiation Process of Human Mesenchymal Stem Cells into Skeletal Muscle Cells

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Objective: Skeletal muscle cells are continually subjected to the production and transmission of biomechanical forces. Biomechanical stimulation during the cell growth contributes to proliferation, differentiation and maturation of skeletal muscle. Human bone marrow mesenchymal stem cells (hMSCs) are able to differentiate into a variety of cell types, including skeletal muscle cells (SMCs), and are potentially a noticeable cell source for muscle regeneration. In this study, it was assumed that the biomechanical forces induced myogenic differentiation process in hMSCs and control expression of myogenic factors transcription.
Materials and Methods: hMSCs were obtained from healthy donors (Iranian Blood Transfusion Organization). Mononuclear cells were subsequently cultured in DMEM-LG containing 10% FBS, 100 IU/mL penicillin, and 100 µg/ml streptomycin for isolation of hMSCs. For applying mechanical loading, hMSCs at 3rd passages cultured on the silicon membrane coated with collagen type I. Uniaxial cyclic loading with frequency of 1 Hz and 10% stretch was applied to cells for 24 hours using the test device designed in National Cell Bank of Iran. We considered four groups in our experiments; a negative control group and three experimental groups. Mechanical loading was applied to (L + F) group after culturing in differentiation medium (DMEM + 5% FBS + 9 ng / ml IGF-I) for four days. F-group was cultured alone in medium containing growth factor. L-group only was subjected to mechanical loading. To check the cellular skeleton structure, actin fibers were stained with Phalloidin. In order to investigate the differentiation of hMSCs into skeletal muscle cells, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was used to examine the expression of some skeletal muscle cell specific genes, including as MyoD (early myogenic differentiation genes) and MyoG (late stage genes of differentiation). Bands obtained with the Image Pro Plus program amounts to quantify transcribed RNA.
Results: Acting filaments arrangement were random in chemical differentiation but after loading, actin filaments tend to approach perpendicular to the loading direction and join together to form thick fibers. RT-PCR results showed that mRNA levels for MyoD, declined in L + F group compared to F group. It could be concluded that with the accelerating early myogenic differentiation trend, transcription of these genes also been faster and came faster into the delayed phase that is the transcriptional induction of MyoG. MyoG RT-PCR results also confirmed this. Significant levels of MyoD and MyoG mRNA in L group showed that mechanical loading can be initiating early myogenic differentiation alone. On the other hand, comparison of the mRNA level between groups F and L, showed that, beginning the process of differentiation in L group occurs slower than group F.
Conclusion: Our results suggest that mechanical loading can alone cause the activation of off cells and their entry is the process of myogenic differentiation. Combined mechanical loading with chemical factors accelerates the process of early myogenic differentiation and have best results for delay stage myogenic differentiation and can be useful for bone tissue repair.
Keywords: Mechanical Loading, hMSC, Myogenic Differentiation

Ps-34: SNAIL Regulates Interleukin-8 Expression, Stem Cell Like Activity, and Umorigenicity of Human Colorectal Carcinoma Cells

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Objective: Some cancer cells have activities that are similar to those of stem cells from normal tissues, and cell de-differentiation correlates with poor prognosis. Little is known about the mechanisms that regulate the stem-cell-like eatures of cancer cells; we investigated genes associated with stem cell like features of colorectal cancer (CRC) cells.
Materials and Methods: We isolated colonospheres from primary CRC tissues and cell lines and characterized their gene expression patters by microarray analysis. We also investigated the biological features of the colonosphere cells.
Results: Expanded CRC colonospheres contained cells that expressed high levels of CD44 and CD166, which are markers of colon cancer stem cells, and had many features of cancer stem cells, including chemo- and radio-resistance, the ability to initiate tumor formation, and activation of the epithelial-mesenchymal transition (EMT). SNAIL, an activator of the EMT, was expressed at high levels by CRC colonospheres. Overexpression of Snail in CRC cells induced most properties of colonosphere cells, including cell de-differentiation. In total, 227 SNAIL-activated genes were upregulated

colonospheres; gene regulatory networks centered around interleukin (IL)-8 and JUN. Blocking IL-8 expression or activity disrupted the SNAIL–induced stem-cell–like features of colonospheres. We observed that SNAIL activated the expression of IL8 by direct binding to its E3/E4 E-boxes. In CRC tissues, SNAIL and IL-8 were co-expressed with the stem-cell marker CD44, but not with CD133 or CD24.
Conclusion: In human CRC tissues, SNAIL regulates expression of IL-8 and other genes to induce cancer stem cell activities. Strategies that disrupt this pathway might be developed to block tumor formation by cancer stem cells.
Keywords: Colon Cancer, Tumor Development, Systems Biology, Gene Regulation

Ps-35: The Effect of The Alignment of Nanofibrous Scaffolds on Mesenchymal Stem Cell Differentiation for Neural Tissue Engineering

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Objective: Nerve tissue engineering is one of the most promising approaches to nerve repair and regeneration. Nanofibrous scaffolds play a pivotal role in tissue engineering. The orientation of nanofibers is one of the important features of a perfect tissue scaffold, because the fiber orientation greatly influences cell growth and related functions in cells such as nerve.
Materials and Methods: In this regard, aligned and random electrospun poly (ε-caprolactone) (PCL) fibers were fabricated to test their potential to provide contact guidance to neural cell from mesenchymal stem cell. Then the chemical and mechanical characterizations of nanofibers were carried out using scanning electron microscopy (SEM), contact angle and tensile instrument. The differentiation of MSCs was carried out using neuronal inducing factors including basic fibroblast growth factor, nerve growth factor and brain derived growth factor in DMEM/F12 media. The differentiation and neurite outgrowth were analyzed by immunocytochemistry and SEM.
Results: Scanning electron microscopy results showed that the direction of cells elongation is parallel to the direction of PCL fibers for aligned scaffolds. The differentiated Mesenchymal cells on nanofiber scaffold were found to express the neuronal proteins, β- tubulin III and Map2 on day 15 after culture. Cell cytoskeleton and nuclei were observed to align and elongate along the fiber axes on aligned nanofibrous scaffolds.
Conclusion: These results suggested that the aligned electrospun nanofibers are capable of controlling the orientation of neurons and acted as a positive cue to support neurite outgrowth.
Keywords: Poly (ε-caprolacton), Mesenchymal Stem Cell, Alignment, Contact Guidance, Neural Tissue Engineering

Ps-36: Differentiation of Bone Marrow Derived Mesenchymal Stem Cells into Osteoblast by Chemical Factors and Mechanical Loading

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Objective: Mesenchymal stem cells are multipotent cells which can produce many lineages. One of these lineages is osteoblast. Osteoblasts are common cell source for regeneration of bone and bone tissue engineering. Mechanical loading plays important roles in regulating the development, function, and repair of bone tissues. We applied two ways to differentiate stem cells into osteoblast; chemical and mechanical inductions. Chemical factors promote osteogenesis and mechanical signals are converted to biochemichal ones. The aim of this study is analysis the effects of chemical factors and mechanical loading on bone marrow derived mesenchymal stem cells (BMSC) differentiation into osteoblast.
Materials and Methods: 4-6 weeks old rats were purchased from Pasteur Institute of Iran (Karaj Unit). Bone marrow mesenchymal stem cells were isolated from femur and tibia. For identification of stem cells, we did pluripotency tests and flow cytometery for BMSC surface CD markers such as CD90 and CD45. Results verified the identity of the cells. Then cells in the 3rd passages were cultured in induction medium contains dexamethasone, ascorbic acid and beta glycerophosphate. Alizarin red staining was done after 7, 10, 14 days. Red granules of calcium was seen. Immunocytochemistry was done to fulfill the work with antibodies against Runx-2 and osteocalcin. Stem cells, cultured on collagen type 1 coated silicon membrane, were subjected to cyclic loading by test device developed in National Cell Bank of Iran. The device applied 3% stretch with 0.3 Hz frequency for 24 hours. After loading, RNA of the cells were extracted. cDNA was synthesized then taqman real time PCR carried out to evaluate the expression of osteogenic marker genes by ABI step one real time device. Primers were designed using primer express software. Selected genes were including core binding factor α (cbfa-1) as early marker of osteogenesis, Osteocalcin (OCN) as late marker and ribosomal protein L-13 (RPL-13) as housekeeping gene. PCR products were loaded on 3% agarose gel for confirmation real time informations.

Results: Real time PCR results showed expression of cbfa-1 and OCN in chemical and mechanical stimuli groups. It was evident that cbfa-1 and OCN expression in mechanical loading tests have increased to chemical induction groups.
Conclusion: This research demonstrates that mechanical stimuli without using chemical factors can have effects on differentiation of bone marrow mesenchymal stem cells into osteoblast and increase this differentiation process to chemical stimuli. So applying mechanical loading can be useful in tissue engineering and regeneration of bone.
Keywords: Mesenchymal Stem Cells, Uniaxial Stretch, Differentiation, Osteogenesis

Ps-37: Effect of Cyclic Stretch and Chemical Factors on Differentiation of Embryonic Stem Cells into Skeletal Muscle Cells

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Objective: Embryonic stem cells (ESCs) are pluripotent cells, which are capable of differentiating into several somatic cell types including skeletal muscle cells (SMCs). Some Environmental factors have been shown to affect the differentiation of ESCs toward the target cells. For instance, it has been observed that, in addition to chemical factors, application of mechanical signals such as cyclic axial stretch, enhances the differentiation of ESCs to skeletal muscle cells .The aim of this study is to study and compare the effect of environmental factors including chemical factors and mechanical stimuli such as cyclic stretch, on the differentiation of ESCs into skeletal muscle cells.
Materials and Methods: After generation of Embryoid Bodies and spontaneous differentiation, mouse ESCs (Royan C4, obtained from Royan institute, Tehran, Iran) were subjected to mechanical and chemical signals, in order to differentiate into skeletal muscle cells. Four test groups were assigned in our research: mechanical, chemical, mechanical-chemical and control groups. In mechanical tests, a cyclic axial stretch device (developed in National Cell Bank of Pasteur Institute of Iran) was used for mechanostimulation . Cells, cultured on collagen type 1 coated silicon membrane, were subjected to cyclic loading .The tests were carried out with 8% load amplitude and 1 Hz frequency for a 24 hour period. In chemical tests, cells were subjected to chemical factors, 5-azacythidine and Horse serum, for 5 days. In chemical-mechanical tests, cells were loaded for 24 hours after they were treated by differentiation medium. We used ESCs in negative control group and mouse skeletal muscle cell (C2C12), National Cell Bank of Iran, in positive control. Then, in order to investigate the differentiation of ESCs into skeletal muscle cells, reverse transcriptase polymerase chain reaction (RT-PCR) was used to examine the expression of some skeletal muscle cell specific genes, including as myogenin (MyoG), myogenic differentiation 1 (Myod1) and myosin heavy polypeptide 2(Myh2). Immunocytochemical staining with skeletal muscle cell antibodies including MyoG, Myod1 and Myh2 were carried out in order to detect the presence of skeletal muscle cell markers.
Results: According to the results obtained from RT-PCR and immunocytochemical staining, chemical-mechanical differentiation test groups, the cells were loaded for 24 hours after they were treated by differentiation medium, showed a higher expression of skeletal muscle cells specific markers including MyoG,Myod1 and Myh2 in comparison to other test groups, including mechanical, and also chemical differentiation ones.
Conclusion: Our results suggest that mechanical loading affects the differentiation of embryonic stem cells into skeletal muscle cells and could be the single differentiation factor. Combination of Chemical factors with mechanical loading, increases the differentiation of embryonic stem cells into skeletal muscle cells and produces functional skeletal muscle cells for engineered tissues.
Keywords: Embryonic Stem Cells, Mechanical Loading, Cyclic Stretch, Skeletal Muscle Cells

Ps-38: Cloning and Assessment of Promoter Activity of Two DNA Regions Upstream of Murine PPARγ Gene

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Objective: Peroxisome Proliferator-Activated Receptorγ (PPARγ) is a nuclear hormone receptor and has two isoforms mouse, PPARγ1 and PPARγ2, which differ in their distribution in tissues and specificity for different ligands. These isoforms are transcribed under regulation of different promoters. Our recent data indicated that the expression level of PPARγ1 but not PPARγ2 was induced after retinoic acid treatment of mouse embryonic stem cells during neural differentiation process. To unravel the molecular mechanism of PPARγ isoforms expression regulation in process of neural differentiation the present study carried out.

Materials and Methods: Bioinformatics studies were performed to identify putative regions responsible for promoters of PPARγ isoforms that indicated these regions contain several transcription factor-binding sites (TFBS). These two putative regions had 3.1 kb and 686 bp length that were selected respectively for cloning. Predicted cloned promoter regions were subcloned into an expression vector upstream of EGFP reporter gene, pDB2. Green fluorescence intensity was quantified by flowcytometry analysis.
Results: Relative activity of these promoter regions were analyzed by green fluorescent intensity of CHO cells transfected by recombinant vectors. These data implicated that activity of PPARγ2 promoter is about 1.17 fold of PPARγ1 promoter. Further experiments on study of these promoter regions like induction of expression by retinoic acid are ongoing.
Conclusion: Two promoter regions of PPARγ isoforms were different based on the length and strength, which implied differential expression patterns of two variants.
Keywords: PPARγ, Promoter, Cloning

Ps-39: Tissue Engineering of Cell-Seeded Polymers for Urethral Support

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Objective: Urinary incontinence has been reported as one of the most common type diseases of the urologic system, a condition that is associated with social impact and a reduced quality of life. Moderate and severe urinary incontinence can be treated by transurethral injection of bulking agents, urethral sling surgery and installation of an artificial sphincter. However, the total therapeutic efficacy of these tools is unsatisfactory. The aim of this work is to make flat circular shaped chitosan hydrogel sheets as cell carriers for using in urological implantation. Muscle-derived stem cell (MDSC) was loaded on the prepared polymeric sheets and the behavior (attachment, expansion and morphology) of the cells on the polymeric hydrogels were investigated in order to check the suitability of the prepared hydrogel sheets for urological implantations. MDSCs are adult stem cells that are good candidate cell types and are preferred to be applied for tissue engineering and clinical usages due to their differentiation potentials.
Materials and Methods: We made flat circular shaped hydrogel sheets made of chitosan based on the appropriate protocols with some modifications to add the required cell-binding sites to improve cell attachment. We used different polymer or cross-linker concentrations to make the hydrogel sheets with various mechanical properties. The suitability of polyelectrolyte hydrogels for ultimate urological applications was assessed by various quantitative and qualitative variables including hydrogel stiffness, easy handling for the surgeon and cell attachment. After washing the prepared hydrogel sheets, we cut each gel by puncher to make circular-shaped polymeric sheets. After immersing the prepared hydrogel sheets in the media for 24 hours, we loaded MDSC cells on each polymeric sheet. The viability of immobilized cells on the hydrogel sheets was investigated by an MTT assay.
Results: In the current study, a model hydrogel substrate based on chitosan polysaccharides in which one can independently vary the hydrogel mechanical properties and the density of adhesion ligands at the material's bulk has been developed. With these studies, we have demonstrated that muscle cell attachment, morphology and proliferation are dependent on the mechanical properties of the hydrogel and the density of adhesion ligands presented at the material's bulk. The observation results showed that cellular morphology was circular at the beginning of incubation and expanded gradually on the hydrogels surface.
Conclusion: These studies lead us to conclude that the material chemistry, material mechanical property and ligand density are important for controlling the function of cells in contact with polymeric matrices.
Keywords: Tissue Engineering, Urology, Muscle-Derived Stem Cell, Chitosan

Ps-40: Differentiated Insulin-Producing Cells from Skin-Derived Stem Cells, Functional or Non Functional?

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Objective: There is widespread interest in developing tissue replacement strategies for the treatment of diseases, such as diabetes and Parkinson disease. Much attention has now been focused on the promising field of embryonic stem cells, but recent studies suggest that skin-derived precursors (SKPs) have an unusually broad differentiation potential. SKPs are more easily accessible than other adult stem cells, which potentially makes them a predominant autologous donor source for stem cell therapy. In the present study, SKPs were isolated and characterized. Functional insulin-producing cells (IPCs) were generated from SKPs under *in vitro* differentiation procedure. Furthermore, we tested the functionality of the *in vitro*-generated IPCs from human skin by measuring insulin release in response to glucose challenge.
Materials and Methods: Sphere colonies of SKPs were converted into IPCs *in vitro*, through extracellular

factor modification and IPCs were analyzed by immunocytochemistry, reverse transcription–polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA).
Results: SKPs could self-assemble to form three dimensional islet cell-like clusters (dithizone-positive) and express insulin. In addition, they expressed multiple genes related to pancreatic β-cell development and function (e.g. insulin, islet-1, NeuroD/beta2, Glut-2 and Nkx2.2), but not other pancreas-specific hormones (e.g. glucagon, somatostatin). Moreover, when stimulated with glucose, these cells synthesized and secreted insulin in a glucose-regulated manner.
Conclusion: Compared to IPCs derived from other adult stem cell sources, we used a simple and accessible way to generate functional IPCs from an abundant autologous stem cell source, SKPs, this hopefully could solve the major problems of availability of donor cells and immunogenic rejection.
Keywords: Differentiation, Skin-Derived Precursors, Insulin-Producing Cells, Autologous

Ps-41: Effect of Feeder Layers on *In vitro* Culture of Bovine Spermatogonial Stem Cells

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Objective: The aim of this study was evaluation and compare the effect of different feeders on *in vitro* short-term culture of prepubertal bovine spermatogonial stem cells (SSCs).
Materials and Methods: The isolated cell suspension containing SSCs were enriched by BSA and gelatin and were cultured in the presence of Glial-derived neuroterophic factor (GDNF), Epidermal Growth Factor (EGF) and Basic Fibroblastic Growth Factor (bFGF), after 7days colonies were harvested and cultured on four different feeders such as STO, MEF, BSC and laminin coated plate. Number and area of colonies were measured in 7th, 11th and 14th days post culture. The expression of SSC markers (α6-Integrin, β1-Integrin, DBA,..) were detected by immunofluorescence assay flow cytometry analysis at day 7 , and Quantitative Real time PCR in four groups at 14 day post culture.
Results: Immuno cytochemical staining revealed that SSC colonies were positive for DBA, α6-Integrin, β1-Integrin and negative for c-kit . In addition the number and area of those colonies were formed on STO feeder were significantly greater in comparison with the other groups . Relative expression of Thy-1 in the STO and then in BSC groups were significantly higher than other group but expression of oct4 was highest in laminin group compare to other groups.
Conclusion: Comparing different feeder layer showed that STO might be a suitable feeder layer for propagation of bovine SCC and increase the number and area of *in vitro* cultured prepubertal bovine spermatogonial colonies.
Keywords: Bovine Spermatogonial Stem Cell, STO, Thy-1

Ps-42: Therapeutic Potential of Long-term Self-Renewable Induced Pluripotent Stem Cells Derived Neural Progenitors for Spinal Cord Injury in Primates

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Globally many thousands of people suffer from acute spinal cord injury (SCI) every year, and SCI still remains a devastating neurologic condition with limited therapeutic opportunities.Many attempts have been made in animals to produce cellular regeneration in the spinal cord using a variety of transplanted cell types. In the other hand human induced pluripotent stem cells (hiPSCs) have led to an important revolution in stem cell research and regenerative medicine.
In order to create patient-specific neural progenitors (NPs), we have established a homogenous, expandable and self-renewable population of multipotent NPs from hiPSCs using an adherent system and defined medium supplemented with a combination of factors and characterized these cells by common cell culture methods and electorphisology.
The established hiPSCs-NPs highly expressed neural cells markers and were continuously propagated for around one year without losing their potential to generate astrocytes, oligodendrocytes, and functional neurons and maintained a stable chromosome number. These NPs represent a potentially useful source of cells for cell replacement therapy after nervous tissue damage.
Keywords: Human Induced Pluripotent Stem Cells (hiPSCs), Neural Progenitors (NPs), Contusion Spinal Cord Model, Monkey, Cell Transplantation.

Ps-43: Retinoic Acid Induced PEP Gene Expression

During Neural differentiation in P19 Cells

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Objective: Peroxisomal matrix protein is mainly expressed in heart, skeletal muscle, and brain tissues. To study the expression of peroxisomal protein (PEP) during neurogenesis, we here employed P19 cells as an *in vitro* model of neural differentiation.
Materials and Methods: Expression pattern of PEP was investigated under distinct steps of differentiation by real time PCR. Time course study of the PEP expression revealed that expression increased prominently during aggregate formation in P19 cells. The expression level of endogenous genes such as MAP-2, PEP, catalase and PEX3 as peroxisomal markers, compared with β-tubulin as housekeeping gene were monitored during different stages of neural differentiation.
Results: The results were semi-quantified and revealed the highest relative expression of PEP in cell aggregates when treated by RA. In contrast, in the absence of RA, the relative expression of PEP was at the lowest level in aggregates and gradually increased in later stages. Unlike expression of PEP and MAP2 which are stage specific dependent, the expression levels of the other two peroxisomal markers, catalase and PEX3, were not stage dependent .
Conclusion: The results of this study showed that elevated level of PEP expression was dependent on RA. A further insight in PEP gene expression, quantitative real time PCR results showed significant difference in PEP expression in presence and absence of RA.
Keywords: Retinoic Acid, Peroxisomal Protein, Neural Differentiation, P19 Cells

Ps-44: Invovlement of PPARγ Activation on Process of Cardiogenesis of Mouse Embryonic Stem Cells

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Objective: To assess the role of PPARγ in process of cardigenesis this study was desined and carried out. Introduction: Mouse embryonic stem cells are pluripotent cells, can differentiate *in vitro* and in vivo into cells representative of the three embryonic germ layers. Peroxisome proliferator activated receptors (PPARs) are ligand-dependent transcription factors which consist of three major isoforms termed α, β/δ and γ. Previous studies have indicated that PPARα activation by related agonist, influeneced the cardiyogenesis process. However the role of PPARγ and its agonist has not been addressed in cardiomycte differentiation so far. In this study, we have assessed the expression of PPARγ during cardiomycte differentiation and effects of its agonist and antagonist on cardiac precursor formation.
Materials and Methods: Embryoid bodies were derived from mouse embryonic stem cells by 2days culture in hanging drops followed by 5 days in suspensions in KDMEM with 15% FCS. For differentiation and formation of cardiac cells, the embryo bodies were plated in KDMEM. To investigate the involvement of PPARγ in cardiac differentiation, its expression was evaluated in embryoid bodies and cardiac cells. Furthermore, to evaluate effects of PPAR gamma agonist and antagonist on cardiac precursor formation, the expression of NKX2.5 specific cardiac precursor was assayed during cardiogenesis.
Results: Data indicated PPARγ expression increased significantly after embroid bodies formation.. Moreover, Expression of NKX2.5 was increased by PPARγ agonist, Rosiglitazone while PPARγ inactivation by PPARγ antagonist (GW9662) decreased its expression when used in 10 μM concentration.
Conclusion: PPARγ activation significantly influences the rate of cardimoygenesis.
Keywords: PPAR Gamma, Cardiogenesis, Embryonic Stem Cell

Ps-45: Functional and Molecular Characterization of Cancer Stem Cells in Human Glioblastoma

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Objective: The commonest class of brain tumours in adults are astrocytomas and the commonest astrocytoma is the malignant glioblastoma (GBM). Median life expectancy in optimally managed patients is 12-14 months with only 25% surviving 24 months. Thus the need for new treatments is an unmet clinical need. In

the last year, the hypothesis that cancer derives from transformed stem cells found its initial confimation in non-solid and solid tumours and according to these findings it has been demonstrated that cancer stem cells exist in human GBM. However, it is not known how they contribute to gliomagenesis and if they can recapitulate the cellular and molecular heterogeneity of GBM. To address this issue, we have characterized cancer stem cells in human GBM at functional and molecular level.
Materials and Methods: Culture propagation, immunofluorescence, *in vivo* experiments and histological analysis were performed as described by Galli et al. Cancer Research 2004 and Piccirillo et al. Nature 2006. Conventional and molecular cytogenetics were performed using QFQ banding and high resolution SNPs arrays. The results were confirmed by using microsatellite analysis.
Results: We report the isolation and genetic characterization of distinct cancer stem cell populations from different areas of the same GBM specimens. These populations retain stem cell features, such as clonogenicity and multipotency and coexist in the same tumour. However, only the cells derived from the core mass appear to possess long-term self-renewal and tumorigenicity. Although derived from the same GBM, these cancer stem cells are genetically different, displaying patterns of karyotype alterations that overlap only partially.
Conclusion: Our findings show that distinct regions of the same GBM specimens contain distinct cancer stem cells which are endowed with different tumorigenicity and independent genomic evolution. Although these cells partially share a common genetic background, they also show peculiar karyotypic abnormalities. Genetic analysis demonstrate that these cells derive from a common ancestor and are responsible for the functional and molecular heterogeneity observed in human GBM. This approach will enable us to understand how the cancer stem cells' model can be applied to GBM and to correlate a functional malignant phenotype with a genetic profile at the final aim to indentify candidate molecular mechanism responsible for gliomagenesis.
Keywords: Brain Tumours, Stem Cells, Tumorigenicity, Glioblastoma

Ps-46: Evaluating the Effects of Growth Factors on Stem Cell Differentiation

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Objective: All stem cells have three general properties: they are capable of dividing and renewing themselves for long periods (prolifration and long-term self-renewal) they are unspecialized; and they can give rise to specialized cell types (differentiation). There are three kinds of stem cells: embryonic stem cells and non-embryonic "somatic" or "adult" stem cells (In some adult tissues, such as bone marrow, muscle, and brain to generate replacements for cells that are lost through normal wear and tear, injury, or disease.). Bone marrow contains at least two kinds of stem cells. First group called hematopoietic stem cells, and second called bone marrow stromal stem cells (mesenchymal stem cells). Stem cells of umbilical cord are the same as hematopoietic stem cells of bone marrow. Stem cells based on ability of differentiation can be classified into four or all the power: Totipotent, Pluripotent, Multipotent, Unipotent. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. But one of advantages of using Adult stem cells is that they are less likely to initiate rejection after transplantation. The most important potential application of human stem cells is the generation of cells and tissues that could be used for cell-based therapies used to replace ailing or destroyed tissue in diseases or traumas. Growth factors exhibit their effects on differentiation and other cellular functions during embryonic development. So they can induce stem cells to become differentiated. In this study, the effects of these factors on differentiation of stem cells into different tissues were evaluated.
Materials and Methods: In these studies, embryonic stem cells (ES) which are pluripotent were derived from rats, canis or human (*in vitro*), and Adult stem cells which are multipotent or unipotent were derived from mesenchymal stem cells of bone marrow or adipose stem cells, or stem cells of umbilical cord. Stem cells were stimulated by Growth Factors such as TGF-β, BMPs, NGF, RA, Shh, PDGF, bFGF, EGF, VEGF, HGF (Hepatocyte GF), To determine the effects of the growth factors on stem cell differentiation into differentiated tissue, expression of specific proteins, antigenic markers and genes were assessed by PCR or immunohistochemical methods, or evaluating differentiation by electronic microscopes, etc.
Results: Evaluations of expressing specific genes and proteins or examination by microcroscopes showed that each of the previously mentioned growth factors activate some molecular mechanisms that have essential effects on differentiation into different kinds of cells.
Conclusion: By expanded studies role of growth factors in differentiation-induction was confirmed by the presence of molecular and antigenic markers in each differentiated cells.
Keywords: Growth Factors, Stem Cells, Differentian

Ps-47: Assessment of PPARγ Isoforms Expression Level during Neural Differentiation of Human Embryonic Stem Cells

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Objective: PPARγ is considered as a nuclear receptor and a transcription factor in many of cells especially adipose tissue. Previous studies have indicated an increment in PPARγ1 expression level during neural differentiation of mouse embryonic stem cells. Bioinformatics data have considered four isoforms for human PPARγ compare with mouse PPARγ that has two isoforms. Thus, the aim of this study was assessment the expression level of different variants of human PPARγ in embryonic stem cells and neural differentiation steps.
Materials and Methods: Treated human embryonic stem cells with noggin and bFGF, were induced to differentiate into neuroectodermal cells with retinoic acid treatment for 6 days. Subsequently, cells were grown without retinoic acid for more 6 days to promote rosette formation. Neural tube-like structures were then emerged after exposure cells with bFGF for extra 6 days. Finally neural tube-like structures were isolated and plated on laminin and polyL-ornithine coated dish for up to 12 days until neural formation completed. RNA extraction carried out from the cells on different stages and cDNA synthesis was performed. Thus obtained cDNAs from human embryonic stem cells, Rosette structures (neuronal precursor cells) and neural cells were applied for real time PCR.
Results: Our result indicated that PPARγ2 do not have any expression in human embryonic stem cells and during neural differentiation steps. Most expression of PPARγ1&3 were observed in neuronal precursor cells (rosette) while showed faint expression in stem cells and neural cells. However, expression of PPARγ4 was in a steady level while its expression level was less than PPARγ 1&3 isoforms.
Conclusion: It can be concluded that PPARγ may exert an influence on neural differentiation of embryonic stem cells and can be considered to play a significant role for neural precursor cell formation.
Keywords: PPARγ Isoforms, Embryonic Stem Cell, Neural Differentiation, Real Time PCR

Ps-48: Construction of a Minicircle DNA Vector In Order Applicable for Generation of Induced Pluripotent Stem Cells

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Objective: Minicircle vectors are DNA molecules that lack bacterial backbone so composed of a eukaryotic expression cassette. Minicircle vectors are capable of higher and longer expression compared to plasmids. Minicircle DNA vectors show rare integration of transgene into genomic DNA. Therefore DNA minicircle will be elutable in cells. thus Minicircle vectors are ideal vectors for generating iPS cells.
Materials and Methods: At the first step, egfp (enhanced green fluorescent protein) CDS was amplified using the plasmid pEGFP-C1 as a template and primers introducing integrase recognition sequences at both of head and tail of amplified fragment. Amplified fragment was then inserted into SnaI site of pBAD.gIIIA. Recombinant plasmid pBAD.EGFP was amplified by transforming into Ecoli cells. At the next step, ΦC31 integrase cDNA was amplified using plasmid pCMV-INT as template and appropriate primers introducing BamHI and SacI sites at head and tail respectively. This amplified fragment was then inserted into pBAD. EGFP , resulting in formation of a new vector termed pBAD. ΦC31.EGFP to generate minicircle DNA. An induction by arabinose in the culture of pBAD. ΦC31. EGFP transfomrmed Ecoli cells, caused generation of minicircle DNA carrying egfp in to the eukaryotic cells.
Results: New recombinant plasmid pBAD. ΦC31. EGFP was constructed successfully and its potency for generation of minicircle DNA in Ecoli cells was verified after induction by arabinose to stimulate the expression of integrase gene.
Conclusion: As minicircle DNA rarely integrate to the host cells, are considered as an ideal system to express the interest genes in the target cells. Recent reports have indicated the application of minicircle DNA for generation of iPS cells. In order to set the same approach for generating iPs, the present study carried out to produce a mother plasmid carrying both of integrase coding gene and a gene marker our data have indicated that such plasmid was successfully constructed and generated a minicicle DNA. This plasmid would be applicable for further studies which is ongoing.
Keywords: Minicircle Vector, Integrase, Recombination, iPs

Ps-49: Enhancement of Proliferation and Neurogenesis of Human Induced Pluripotent Stem Cell-Derived Neural Progenitors by Application of a Small Molecule

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Objective: Neural progenitors (NPs) have the ability to self-renew, and differentiation to both neuronal and glial lineages. Therefore, these cells have capability for cell therapy in neurodegenerative diseases. One challenge in this regard is their expansion and directed differentiation. To increase the proliferation and differentiation of NPs, we evaluated the influence of inhibition of glycogen synthase kinase 3 (GSK3) by CHIR99021.
Materials and Methods: Human NPs were derived from human induced pluripotent stem cells (hiPSC-NPs). The small molecule CHIR99021 was used to inhibit GSK3 and for study of its cross-talk with other pathways small molecules DAPT, XAV939, and pifithrinα applied to inhibit notch, β-catenin and p53 signalling, respectively. Proliferation of the cells determined by MTT assay and flowcytometry for Ki67. QRT-PCR and Immunofluorescence staining were used for mRNA and protein quantification.
Results: MTT and Ki67 analyses revealed that CHIR99021 increased the proliferation of hiPSC-NPs across 10 passages and there was no chromosome instability as detected by karyotype analysis. Pifithrinα had no major effect on the proliferation while DAPT and XAV939 decreased the proliferation. The expression of HES1, HES5 (Notch related transcription factors) and CYCLIND1, C-MYC (β-catenin related transcription factors) increased significantly in the CHIR99021 treated NPs compared to DAPT and XAV939 groups. CHIR99021 treatment in the differentiation process impaired gliogenesis and enhanced neurogenesis Whereas DAPT treatment had no effect on the differentiation in the CHIR99021 treated hiPSC-NPs and XAV939 could blocked neural differentiation. Expression of neural genes such as NGN3 and MAP2 and also dopaminergic genes such as TH,LMx1A,LMx1B and NURR1 were increased upon CHIR99021 treatment.
Conclusion: Our results determined GSK3 inhibition could promotes proliferation of hiPS-NPCs by β-catenin stabilization and notch activation. Also CHIR99021 improved neural differentiation through dopaminergic fate by β-catenin stabilization and in turn expression of proneural genes such as NEUROD1 and NGN3 and increased the expression of dopaminergic genes.
Keywords: Neural Progenitor Cells, Small Molecules, Glycogen Synthase Kinase3, Differentiation

Ps-50: Stem Cells Transdifferentiation after *In vitro* Transplantation in Neural Tissue

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Objective: The functional rehabilitation of damaged retina in different cases of pathology with stem cells transplantation is very actual nowadays in ophthalmology and can be used as a model for studying cell engrafting in brain tissue. In vivo studies can't show us all changes in stem cells and recipient interactions after transplantation and it is impossible to obtain enough information about transplanted cells behavior during experiment. That's why it is necessary to design *ex vivo* models allowing registering the individual cell behavior after transplantation and clear visualization of their changes. *In vitro* injection of xenogeny cells into 3D rat retina culture seems to be an attracting method in this case.
Materials and Methods: 7-day old rats' retina explant culture (DMEM/F12 with 20 ng/ml FGF and EGF, 7% FCS, B12 и N2 supplements) damaged with laser Zilos-tk (300mW, 1000 mc) had been used. Bone marrow stromal cells (MMSC) of C57BL/6-Tg(ACTB-EGFP)/Osb/J GFP+ mice had been transplanted in them (300-300000 cells in 0,2 mkl) *in vitro*. AFM data were obtained from Atomic force microscope Solver BIO Olympus with the scanning field 100 × 100 × 7mkm^3. Data analysis was held by programs Nova (HT-MDT) and STATISTICA 8.0. The MMSC reaction on external irritation was estimated by fluorescent die RH 795 after electro stimulation on ASL-1 device.
Results: After MMSC transplantation two populations of small (≈15mkm) rapidly migrating cells and almost motionless big (≈30mkm) cells were remarkable among injected MMSC. On the 3-d day after injection in intact retina MMSC changed their morphology with spreading long branched outgrowths but were still negative for neural and glia cell markers (β-III-tubulin and GFAP). And analogous morphology changes of transplanted MMSC in damaged retina were observed after 24 hours. MMSC injection had an expressed neuroprotective effect on retina. ASM assay had showed significant difference ($p<0.01$) between thickness of glia and endothelial retina cells processes and neurites and neuro-like transplanted MMSC processes. Also it was showed that MMSC form synapses up to 2.5 ± 0.06 mkm in diameter on the 4th day after transplantation. After electro stimulation (20 V, 0.5 Hz, 200 ms) clear depolarization of retina neurons and their processes was detected. It was shown that some of those GFP+ MMSC witch had been changed their morphology after transplantation in retina explants to neuro-like were able to depolarize after exogenic stimulation. Because of absence of transplanted GFP+ MMSC and retina neurons fusion, detected by fluorescent DiI staining it is possible to suppose that there is a small MMSC subpopulation that is able to neuronal

transdifferentiation.
Conclusion: The effective *ex vivo* model of explantation culture for detecting cell migration, differentiation and communication processes in recipient tissue directly after transplantation was developed and successfully applied for MMCK *in vitro* transplantation. It was shown that some subpopulation of MMSC had an ability to neural transdifferentiation and using this method it is possible to optimize clinical transplantation protocols and makes them more effective.
Keywords: Stem Cell Transplantation, Retina Damage Model, Stem Cells Transdifferentiation

Ps-51: Knock-Down of Nucleostemin Gene Expression by RNAi Induced Differentiation and/or Apoptosis in Leukemic Stem Cell Models

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Objective: Leukemia is an important disease with stem cell origin which arises in CD34+ cells. Identification and targeting of signaling pathways involved in proliferation, differentiation and apoptosis of leukemic stem cells are new frontiers in treatment of CML patients. In this case, nucleostemin (NS), a novel nucleolar GTP-binding protein, plays a critical role in controlling cell-cycle progression, self-renewal and proliferation of both stem cells and cancerous cells. Expression of NS gene in leukemic stem cells has been demonstrated, but there is no comprehensive research in NS mechanisms of action yet. The aim of the present study was to introduce NS-siRNA into human leukemic stem cell models (K562 and NB4) and investigated effects of NS gene silencing in growth, differentiation and apoptosis of leukemic stem cells.
Materials and Methods: NS specific double-stranded small interfering RNA (NS-siRNA) was transfected into leukemic stem cell models (K562 and NB4). Changes in NS gene expression pattern evaluated by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). [3-(4, 5'-dimethyltiazol-2-yl)-2, 5-diphenyltetrazolium bromide] colorimetric assay (MTT) and trypan blue exclusion test were used for cell proliferation and viability evaluation. Flow cytometery was used for evaluation of cell-cycle states of treated cells. Differentiation was studied using Wright-Giemsa staining and latex particle phagocytic activity. Apoptosis was studied using fluorescent microscopy.
Results: The results showed that NS gene was highly expressed in K562 and NB4 cells. After 72 hours transfection of 200 nM siRNA, growth was inhibited up to 30-40% in both cell lines. In addition, viability was significantly reduced up to 30-40% after 72 hours in K562 cells. But, no substantial reduction on viability of NB4 cells was observed. Mechanistic study showed NS-siRNA treatment of K562 cells resulted in G1 cell-cycle arrest followed with apoptosis after longer transfection times. Whereas, differentiation characteristics such as decrease in nuclear cytoplasm ratio and ingestion of latex particles were observed in NB4 cells. Overally these results suggested that apoptosis in K562 and differentiation in NB4 were ultimate effects of NS inhibition.
Conclusion: Based on apoptosis and differentiating effects of NS-siRNA in human leukemic stem cell models, NS may have potential application in molecular therapies based on induction of differentiation and apoptosis in leukemic stem cells of leukemia patients.
Keywords: Apoptosis, Differentiation, Leukemic Stem Cells, Nucleostemin

Ps-52: Stem Cell Characteristics of Prostate Cancer Cell Lines

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Objective: Recent studies indicate the presence of a small, stem-like cell population in several human cancers that is crucial for the tumour (re)population. Six established prostate cancer (PCa) cell lines - DU145, DuCaP, LAPC-4, 22Rv1, LNCaP, and PC-3 - were examined for their stem cell properties *in vitro*.
Materials and Methods: The colony-forming efficiency and self-renewal ability of morphologically distinguishable holoclones and paraclones were tested with low-density plating and serial passaging. Expression of the putative stem cell marker CD133 and breast cancer resistance protein (BCRP) was examined with flow cytometry, and immunohistochemical stainings were made for CD133, a2-integrin, nestin, BCRP, cytokeratin 5 (CK5), and cytokeratin 18 (CK18).
Results: Five out of six cell lines formed clear holo-, mero-, and paraclones. Unlike paraclones, we can maintain DU145 holoclones in culture for several passages, which is indicative of self-renewal ability. Using fluorescenceactivated cell sorting (FACS) analysis only in DU145 cells, a small fraction (0.01%) of CD133+ cells was detected. CD133+ cells; however, like DU145 BCRP+ (0.15%) cells, they were not more clonogenic, and they did not show more holoclone formation than the marker-negative cells or unselected cells. Immunohistochemistry revealed a2-integrin and BCRP as potential stem cell markers and CK5 with the combination of CK18 to distinguish transient amplifying cells.
Conclusion: These results indicate the possible presence of stem-like cells in several established PCa cell lines. CD133 selection does not enrich for stem-like cells in PCa cell lines.
Keywords: Prostate Cancer, Stem Cells, CD133

Ps-53: Efficient and Expedited Derivation of Induced Pluripotent Stem Cells in Stirred Suspension Bioreactors

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Objective: Induced pluripotent stem cells (iPSCs) have been previously developed by reprogramming somatic cells following expression of defined transcription factors. The reprogramming of somatic cells through this technology is a valuable tool to identify the mechanisms of pluripotency. From the clinical perspective, a fundamental prerequisite is to generate relevant cell numbers through an economically viable bioprocess. Although routinely used, the static derivation of iPSCs is inefficient and can only support pre-clinical research projects. The robust and expedited generation of large cell numbers required for therapeutic interventions would only be feasible within a controlled bioprocess. In this study, we examined whether stirred suspension bioreactor (SSB) culture presents a selective advantage in iPSC derivation.
Materials and Methods: We transduced mouse embryonic fibroblasts (MEFs) with retroviral vectors of the three reprogramming genes (Oct4, Sox2 and Klf4). Two days after transferring to a 100mL SSB at 100RPM, the MEFs formed aggregates, which were morphologically similar to ESC aggregates. Derived aggregates were characterized *in vivo* and *in vitro*.
Results: Here, we report that iPSCs can be generated quickly and efficiently within SSBs. We further demonstrate that fully reprogrammed iPSCs can be derived in SSBs in 10 days. The resulting Bioreactor-derived iPSCs (BiPSCs) resembled embryonic stem cells in their *in vitro* and *in vivo* characteristics including gene expression and differentiation potential. BiPSCs also display a normal karyotype, contribute to teratomas, produce viable chimera and display germ line transmission competency. This study clearly demonstrates that fibroblasts can be reprogrammed in suspension efficiently in large quantities and in a fraction of the time compared to static derivation. When we examined the nuclear translocation of β-catenin in ESC, using the TCF/LEF GFP reporter system, we observed that considerably more β-catenin resides in the nucleus of cells exposed to increased shear stress. This suggests that suspension culture provides a selective advantage in enhancing iPSC generation partly by inducing nuclear β-catenin activation.
Conclusion: Our results show for the first time that fibroblasts can be efficiently reprogrammed in SSBs. We propose that liquid shear stress plays an important mechanistic role in bioreactor induced pluripotency. Combined with new methods of reprogramming that only use epigenetic reprogramming factors, our BiPSC technology has the potential to accelerate and standardize iPSC research, bringing it to clinical application more quickly.
Keywords: iPS Cell, Cellular Reprogramming, Stirred Suspension Bioreactor

Ps-54: Impact of Stirred Suspension Bioreactor Culture on the Differentiation of Murine Embryonic Stem Cells into Cardiomyocytes

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Objective: Embryonic stem cells (ESCs) can proliferate endlessly and are able to differentiate into all cell lineages that make up the adult organism. Under particular *in vitro* culture conditions, ES cells can be induced to differentiate into cardiomyocytes, albeit the efficiency is low and the underlying mechanism of differentiation is not fully understood. Stem cell therapy using ESC derived cardiomyocytes offers the possibility to replace damaged cardiomyocytes in a patient with cardiovascular disease; however, these future clinical applications will demand large numbers of functional cardiomyocytes. It has been previously demonstrated that ESCs can be expanded and differentiated in stirred suspension bioreactors, but if we utilize these types of systems we have to be cognizant of the mechanical forces acting upon the cells. The effect of mechanical forces and shear stress on ES pluripotency and differentiation has yet to be clarified. Therefore, we have undertaken the present study which involves: (i) induction of ESC derived cardiomyocytes in stirred suspension bioreactors followed by (ii) investigation of the effect of shear stress on pluripotency of ESCs during differentiation.
Materials and Methods: Treatment of embryoid bodies (EBs) with 10mM AA and 0.5% DMSO from day 3 markedly increased the number of beating EBs which displayed spontaneous and cadenced contractile beating on day 11 in the bioreactor. The properties of derived-cardiomyocytes were analyzed by immunocytochemistry, Quantitative PCR, transmission electron microscopy (TEM), Flowcytometry and positive and negative chronotropical drugs.
Results: Interestingly, our results demonstrated that the pluripotency markers including Oct4, Nanog and Sox2

were expressed during differentiation in a subpopulation of cells even after one month of suspension culture in the absence of LIF and negative drug selection.
Conclusion: This study demonstrates that although cardiomyocyte differentiation can be achieved in a bioreactor culture system, forces within this system appear to maintain a subpopulation of cells in a transient pluripotent state. Despite the fact that stirred suspension cultures are very useful for the generation of a large number of undifferentiated cells, we have found that the addition of medium enhancers is not adequate to force complete differentiation of the population in the suspension bioreactor, due to the effect of shear stress on the cells. By elucidating the exact mechanism(s) by which the shear forces may contribute to pluripotency and prevent differentiation, we will be able to create an efficient environment for the production of large quantities of pluripotent stem cells, iPSCs, as well as differentiated cells, which is important for future application of these cells in regenerative medicine.
Keywords: Embryonic Stem Cell, Cardiomyocytes, Differentiation, Stirred Suspension Bioreactor

Ps-55: The Expression of CD44 in Cord Blood CD34+ Cells Affected by Substance P

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Objective: Modulating the expression of cell adhesion molecules on hematopoietic stem cells (HSCs) of cord blood (CB) may help to improve homing process of CB stem cells. Microenvironment and released mediators are major factors in the process of engraftment. Based on evidence concerning the existence of substance P (SP) receptor on cord blood CD34+cells, we aimed to explore effects of SP on CD44 expression on CB-HSC.
Materials and Methods: CD34+cells purified from CB, were cultured in a serum-free liquid culture system. Different concentrations of SP were used in combination with cytokine cocktail of SCF, FL, TPO, IL3 and IL6. Control groups were treated with cytokine cocktail. CD44 expression was enumerated by flowcytometry.
Results: Our results show significant increase in frequency of SP treated cells at 10-7M and 10-11 M following 7 days cultivation as compared to control group. Additionally median flowcytometric intensity of CD44 at corresponding culture period and SP concentrations were significantly increased compare to freshly purified CD34+cells at day 0.
Conclusion: Our findings suggest that SP neuropeptides could act as a novel supplement for cytokine cocktails to maintain or increased CD44 expression which could be beneficial in homing of cells in transplantation.
Keywords: SP, CD44, Cord Blood, Stem Cell

Ps-56: Growing Vascularised Thick Tissue in a Novel Designed Bioreactor

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Objective: Organ transplantation as a medical treatment is a last resort, life saving option for some injuries and diseases. A major problem, however, is the scarcity of donor tissue and organs. Current use of engineered human tissue is limited to thin tissues (100-200 μm). For other thick tissues, fully functional blood vessels must be created. Human mesenchymal stem cells (hMSCs) are known for their ability to self renew, undergo cloning and differentiate into other cells. Moreover, the ability of MSCs to mediate immunosuppression gives them an important role in limiting the rejection of foreign tissue in regenerative medicine.
Materials and Methods: In this project, 5% scaffolds constructed from Poly lactic acid (PLA) by a thermally induced phase separation (TIPS) technique have been utilized as a biodegradable 3D structure. PLA was dissolved in ethylene carbonate (EC) at 70°C. The polymer solution was then cast in a cylindrical mould. Next, crystallisation was induced manually at room temperature. The solidified polymer solution was then kept at 4°C cold room overnight before leaching. Next, the solidified PLA/EC was leached in pre-cooled de-ionized (DI) water at 4cC for 2-3 days to remove EC. Finally, the scaffold samples were recovered from the DI water and transferred into a vacuum container overnight. These fabricated scaffolds were then cultivated with hMSCs. Initially, static culture conditions were tested and then hMSCs were seeded within the bioreactor to compare the results of static and dynamic culture conditions.
Results: Thick scaffolds were seeded with hMSCs in 3D static conditions. The media was changed every 2-3 days over a period of 2 weeks. MTT and confocal were indicated that the number of viable seeded cells with metabolic activity were higher on the top and sides of the scaffold in comparison with the bottom in static culture conditions. Then, thick scaffolds were seeded in 3D dynamic conditions within the bioreactor, and were cultured for 2 weeks. MTT and confocal staining revealed uniform seeding of the hMSCs; this presumably resulting from the continuous drip feeding loop within the bioreactor in dynamic culture conditions. Thin scaffolds (were also examined when seeding the hMSCs in 3D static conditions and were cultured for

8 weeks. Differentiated osteogenic cells were stained with Alizarin Red-S. These cells actively produced extra cellular matrix (ECM), albeit the lower number of visible nuclei suggests that the cell density is still quite low. Finally, quantitation of hMSC differentiation to osteogenic cells was confirmed by quantitative RT-PCR. RNA concentrations from 64 to 200 ng/µl were extracted from the hMSC cells in the scaffold using Trizol. RT-PCR subsequently revealed the presence of osteogenic markers; Osteocalcin, Collagen 1A and RUNX2.

Conclusion: The goal of this research is to facilitate culture of hMSCs seeded throughout a 3D scaffold that can be fed with nutrients using the media with the established growth factor and hormone cocktails. At the same time, a suitable environment can be provided to allow the sprouting and growth of a blood vessel network which may lead to the production of vascularised 3D hMSCs seeded scaffolds with enhanced thickness (1 cm).

Keywords: Organ Transplantation, Human Mesenchymal Stem Cells, Tissue Engineering, Vascularisation, Bioreactor

Ps-57: Treatment of Bone Defects with Mesenchymal Stem Cells (MSCs) Graft in Rabbit Animal Model

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Objective: Cell grafting ia an alternative for treatment of bone defects. Broad bone defects due to infection or trauma egarded is a chief problem in orthopedic surgeries.

Materials and Methods: In the present reserch, a $3mm^2$ tibia defect in male rabbit was repaired by using mesenchymal stem cells (MSCs) and osteoblasts cells graft homologouse seeded on hydroxyapatite- tricalcium phosphate (HA-TCP, n = 6) and was compared by treated with HA -βTCP alone (n = 6). Adverse feet no treatment for the defects as controls. In 3 experimental groups new bone formation was investigated after 6 weeks .Osteojenic differentiation marker expressions assessed by amplication via the RT-PCR such as type I collagen and osteocalcin, for the mesenchymal stem cells and the osteoblast cells seeded on HA -βTCP.

Results: The mean defects healing based on the histological studies in different groups than the control group was significant ($p<0.001$). The results from express bone specific genes, collagen type I and osteocalcin expression in the osteoblast cells / HA- TCP group (n = 4) and no expression of these two genes in group with mesenchymal stem cells (n = 3) showed.

Conclusion: According to the results defects created in rabbit tibia using a synthetic scaffold HA / TCP by osteoblastic cells, mesenchymal stem cells well was repaired. Although the amount of healing were different and even in cell-free samples there was nevertheless showed that the osteoblastic cell transplantation provides a better repair.

Keywords: Osteoblasts Cells, The Mesenchymal Stem Cells, Hydroxyapatite - Tricalcium Phosphate Scaffold, Bone Defects, Treatment

Ps-58: Expression of GATA-4, Nkx2.5, and α-MHC Genes of Cardiomyocytes Derived from Human Embryonic Stem Cells

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Objective: Cardiomyocytes derived from human embryonic stem cells (hESCs) could be useful in restoring heart function after myocardial infarction or in heart failure. Cardiovascular system is the first system to be established during early embryogenesis. The hESCs can differentiate *in vitro* into spontaneously contracting cardiomyocytes and produce a model to investigate the early developmental stages of this system. To fully use the potential of the cells, they need to be extensively characterized, and the regulatory mechanisms that control hESCs differentiation toward the cardiac lineage need to be better defined.

Materials and Methods: After removing of cells from their feeder layer, hESCs create embryoid bodies (EB) which, when plated, develop areas of beating cells in the EB. The expression pattern of several cardiac-specific genes such as GATA-4, Nkx2.5, and α-MHC were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) of RNA samples taken at five time points during the development of EB in suspension (days 2, 4, 7, 8, 10, 14, and 20 of culture). For semi-quantitative analysis, the PCR results were measured by densitometry and normalized to GAPDH generated from the same sample.

Results: There was an enhanced expression of GATA-4 which is a transcription factor known for its central role in regulating different genes involved in the differentiation and development of the heart. Dramatic increase was started in expression of GATA-4 from day 7 in EB in suspension, remaining high-expressed during the following days, and decreasing after day 19. The Nkx2.5 gene expression was first specified in 4-day-old EB and was significantly increased by day 14. The expression of α-MHC gene was also investigated in pulsating EB and a significant decrease was examined in 43-day-old pulsating EB compared to those of 3 days of pulsations.

Conclusion: Following the developmental course of human embryos, cardiomyocytes obtained in culture from

hESCs and therefore might be useful as an effective model system for understanding the developmental processes and functioning of the human heart. They may also provide potential tools in fields such as drug development and testing, early heart development and clinical treatment based on cell transplantation and tissue engineering.
Keywords: Cardiomyocytes, Embryoid Bodies, Gene Expression, Human Embryonic Stem Cells

Ps-59: Don't Waste Pulp Polyps; They May Be Reach Sources of Dental Pulp Stem Cells

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Objective: Adult stem cells have been identified in a variety of tissues as a population of multipotential self renewing cells. Dental pulp stem cells (DPSCs) in comparison with other sources of adult stem cells such as bone marrow have shown to have more replicative potentials while having the same differentiation capacity. Third molar teeth and deciduous teeth have been used widely for isolation of DPSCs. The Goal of this study was to assess possibility of isolation, differentiation and characterization of dental pulp stem cells from pulp polyps which are considered as pathologic tissues and are frequently thrown away in clinics. The pulp polyp, or chronic hyperplastic pulpitis, is produced due to the inflammatory proliferative response in a tooth with a very good blood supply.
Materials and Methods: Pulp polyps of four permanent molar teeth achieved through simple curettage of pulp chamber. The tissues were digested by collagenase and dispase. The obtained single cell were harvested and cultured. After 3 passages, cells underwent flowcytometry for CD73, CD34, CD146, CD45, CD14, CD90, CD105, STRO1, CD44, CD166, HLA-DR.
Results: Cells isolated from pulp polyps displayed similar features as bone marrow derived mesenchymal stem cells (BM-MSC) do. They were fusiform fibroblast-like plastic adherent cells. Cells were more than 90% positive for CD73, CD166, CD90, CD44 and negative for CD34, CD45, and CD14. But only half of the cells were positive for CD146, STRO1, CD105 and HLA-DR.
Conclusion: Pulp polyps are easily accessible non-invasively acquired tissue resource which may contain frequent number of DPSCs. Since the acquired population of cells showed a heterogeneous cell surface antigen panel, there is a possibility for presence of fibroblasts among these cells which needs further analysis with differentiation studies.
Keywords: Adult Stem Cells, Dental Pulp Stem Cell, Pulp Polyp, Chronic Hyperplastic Pulpitis

Ps-60: Actinidin: A Proper New Collagenase For Isolation of Skin Stem Cells

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Objective: Actinidin is a cysteine protease abundant in Kiwifruit. This enzyme is known as a meat-tenderizing protease. After purification the enzyme was used for degradation of dermis and isolation and culture of the skin-derived precursors (SKPs). SKPs are a type of progenitor cells isolated from mammalian dermal tissue and can be differentiate to neural and mesodermal lineage *in vitro*. Although our results revealed that actinidin can hydrolyze and degrades native collagen generally, it specially hydrolyze collagen types I and II at neutral and alkaline buffers. Furthermore, actinidin isolated intact SKPs as same as collagenase type H (which routinely uses for isolation of stem cells from dermis and other tissues) with viability more than 90%. Also we tested this new collagenase for isolation of different cell types for primary culture such as: Hepatocytes, Thymic epithelial cells, Umbilical vein endothelial cells and etc.
Materials and Methods: We purified actinidin from kiwifruit by salt precipitation and ion exchange chromatography. Collagenolytic effect of the purified enzyme was tested in four different buffer systems. Human foreskin samples were used to obtain SKPs after digestion of dermis by actinidin (in different concentration). SKPs viability was tested by trypan blue assay. And SKPs was confirmed by cell surface marker in immunocytochemistary assay.
Results: Our results showed that actinidin can hydrolyze collagens of dermis and therefore release of cell from extra cellular matrix, it specially hydrolyze collagen types I and II at neutral and alkaline buffers. SKPs that isolated were cultured successfully and their viability compare to SKPs that are isolated by other common collagenase was the same.
Conclusion: Actinidin can be a good, cheap and available source of plant collagenase for cell isolation and also stem cell research instead of common animal and bacterial collagenase that are expensive and hard to obtain. Isolation properties of actinidin is the same as other collagenase and in some cases better than them.
Keywords: Actinidin, Collagenase, Skin, Dermis, Stem Cells

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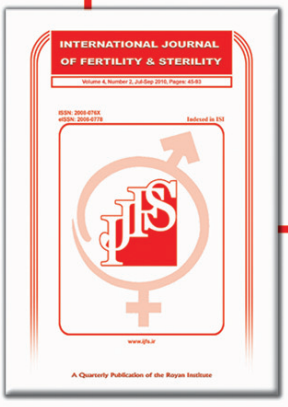

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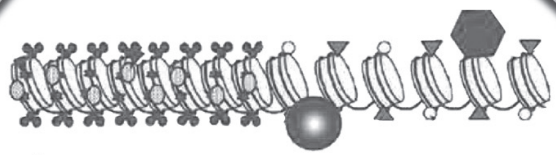

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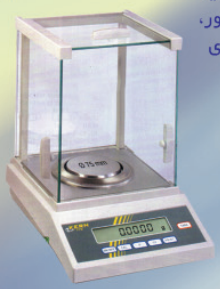

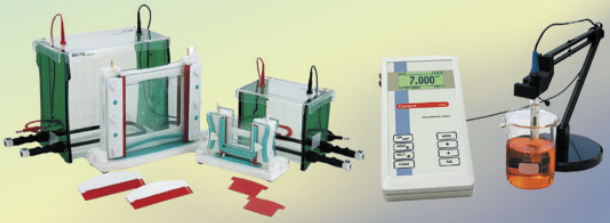

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با مجوز از اداره کل تجهیزات پزشکی و وزارت صنایع و معادن استان تهران

www.jaltajhiz.net info@jaltajhiz.net

تولیدات شرکت ژال تجهیز :

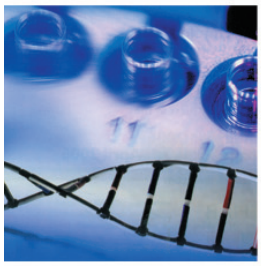
۱- لامینارفلو انواع کلاس‌های ۱، ۲ و ۳ - IVF, PCR
۲-لامینار فلو کلاس ۲ مجهز به سیستم Cooling
۳- دیپ فریز ۸۶- و ۸۰- درجه سانتی‌گراد- ایستاده و صندوقی
٤- فریزرهای ۲۰- و ٤۰- درجه سانتی‌گراد (فریزر نگهداری پلاسما)
۵- ژرمیناتور - اتاقک تست پایداری
۶- اینکوباتور CO_2، مجهز به سیستم رطوبت
۷- شیکر اینکوباتور یخچال‌دار در اندازه‌های ۲۰ و ٤۰ و ۵۰ لیتر
۸- رولر اینکوباتور یخچال‌دار
۹-اینکوباتور یخچال‌دار
۱۰- یخچال بانک خون
۱۱-یخچال آزمایشگاهی
۱۲- آون ۲۵۰+ درجه سانتی گراد
۱۳- فریز درایر (جهت ویال و آمپول)
- مشاوره و اجرای کلیه امورآزمایشگاهی و تحقیقاتی
- دستگاه‌های فوق در مدل‌ها و اندازه‌های مختلف تولید می‌شود.

New JTLVC2X لامینار فلو - عمودی - کلاس ۲
با سیستم سیرکولیشن - درب شیشه‌ای برقی
آلارم هشداردهنده اشباع فیلتر و وضعیت درب
کنترلر دیجیتال

دیپ فریز ایستاده
۸۰- درجه سانتیگراد
Ultra low temperature
freezer (upright - 80°c)

JTBL560
بانک خون

اینکوباتور شیکر یخچال‌دار
مدل JTS-L50
دو طبقه - قابلیت شیک کردن ۵۰ لیتر مایع
دامنه درجه حرارت ۵+ الی ۵۰+ درجه سانتیگراد
کنترل ٤۰ الی ۳۰۰ دور دقیقه



اینکوباتور یخچال دار
مدل JTIL200

شرکت ژال تجهیز (با مسئولیت محدود)

آدرس کارخانه: کرج- کمالشهر- ضلع غربی شرکت رنوس - خیابان صفا- بن بست اول سمت چپ - درب دوم سمت چپ - پلاک ۲

تلفن: ۰۲۶۱-۴۷۰۶۱۱۰/۱۱-۴۷۰۷۹۴۸/۴۹-۴۷۰۴۴۴۰ تلفکس: ۰۲۶۱-۴۷۰۹۸۲۸ همراه: ۰۹۱۲-۱۹۸۵۱۲۵ ۰۹۱۲-۶۶۱۲۵۶۶ ۰۹۱۲-۱۶۹۶۳۵۳ ۰۹۱۲-۶۶۱۷۱۲۰

PerkinElmer
precisely.

DELFIA Xpress

سیستم کاملاً اتوماتیک غربالگری جنین
در سه ماهه اول و دوم بارداری جهت تعیین ریسک
ابتلا به بیماریهای تریزومی ۲۱، تریزومی ۱۸،تریزومی
۱۳ ، ONTD (نقص لوله عصبی) و pre-eclampsia.
قابلیت آنالیز ۴۰ نمونه در ساعت
ظرفیت ۳۰ نمونه در هر بارگذاری دستگاه
نرم افزار تعیین ریسک
- مارکر های قابل اندازه گیری :

hAFP
PAPP – A
UE3
hCG
Free hCGβ
PlGF
*PP13

*Under Development

AutoDELFIA:

سیستم Immunoassay کاملاً اتوماتیک جهت تست های غربالگری و تشخیص های روتین در فیلدهای :

- غربالگری سه ماهه اول و دوم بارداری
- غربالگری نوزادان
- تیروئید
- آنمی (کم خونی)
- آنکولوژی
- دیابت
- بیماری Celiac
- باروری
- و ...

- دارای دو بخش Sample Processor و Plate Processor.
- قابلیت پذیرش ۴۳۲ نمونه سرمی در ۳۶ rack .
- استفاده از بارکد برای شناسایی نمونه ها .
- قابلیت اندازه گیری حداکثر ۸ تست بر روی هر نمونه .
- ظرفیت پذیرش ۱۲ microtitration plate ۹۶ خانه ای .
- قابلیت استفاده از نرم افزار Elipse برای تفسیر نتایج و ارائه‌ی ریسک آنالیت‌ها در سه ماهه‌ی اول و دوم بارداری و همچنین قابلیت اتصال به نرم افزار LIS.
- قابلیت اتصال به سیستم DBS Puncher برای نمونه های DBS (قطره خون خشک).

شرکت جم آریا فن آور

آدرس: تهران، شهرک غرب، بلوار دریا، خیابان گلها، خیابان توحید ۵، پلاک ۱۴، واحد ۴
تلفن: ۸۸۳۸۵۲۰۰ فاکس: ۸۸۳۸۵۲۰۱ ص.پ:۴۷۹-۱۴۶۵۵ info@jaicoltd.com

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فناوران زیستی امید

دستگاه **Ettan IPGphor3** جدیدترین دستگاه ساخت کمپانی فارماسیا آمرشام می باشد .
این دستگاه با امکاناتی از جمله سینی هایی از جنس سرامیک برای مرحله فوکوسینگ و یا **IPG Box** که انجام مرحله **Rehydration** را بدون نیاز به روغن امکان پذیر می نماید و یا سینی یک تکه برای هر **5** سایز ژل های **7،11،13،18،24 cm** که نیاز به خرید سینی های مختلف برای سایزهای مختلف را مرتفع می سازد انجام آزمایشهای الکتروفورز دو بعدی را بسیار آسان ساخته و کیفیت نهایی تست را در حد قابل ملاحظه ای ارتقء می دهد .
همچنین همراه با دستگاه نرم افزاری جهت طراحی تست و نهایتاً ذخیره اطلاعات آزمایش انجام شده به صورت اطلاعات آماری و یا نمودار ارائه می شود .
مشخصات :

Capacity Maximum of 12 Strips
Run Voltage :10-10000V-in 10-7 Steps
Run Current :0-1.5m A((دستگاه امکان انتخاب جریان را نیز به شما می دهد

Ettan DIGE Imager

ImageScanner III

SE 600 Ruby

CYTOGENETICS

Nucleofector® Technology – two modular devices

آدر س: تهران – بزرگراه چمران – پل مدیریت –
بلوار دریا -بعد از چهار راه قدس - خیابان رامشه -
توحید 5 غربی – پلاک 14–واحد 2
تلفن : 88385195 فکس : 88385196

email:info@hbiotechnology.com

شرکت ژن زیست یاخته (با مسئولیت محدود)

وارد کننده و توزیع کننده مواد ، معرفها و محیطهای کشت سلولی آزمایشگاههای سلولی ـ مولکولی ، بیوتکنولوژی ، سیتوژنتیک و سلولهای بنیادی StemCell

---

نماینده انحصاری شرکتهای

CELLNTEC advanced cell systems
سوئیس

تولید کننده سیستم های پیشرفته کشت سلولی و طراحی محیط های کشت اختصاصی برای کشت های تک لایه ای ، دو بعدی و سه بعدی . شامل محیط های کشت اختصاصی سلولهای قرنیه ، دهان ، پستان ، پروستان ، مجاری تنفسی ، مجاری ادراری و تناسلی

bio west فرانسه

بزرگترین تولید کننده سرم های جنین گاوی FBS و انواع حیوانی محیط های کشت سلولی آماده و پودر و معرفهای بیوشیمیایی

CYTOGEN آلمان

بزرگترین تولید کننده محیط های کشت سلولی آزمایشگاههای سیتوژنتیک
AmnioGrow for Prenatal , LymphoGrow for Postnatal
MarrowGrow

WAK - Chemie Medical GmbH

بزرگترین تولید کننده محلولهای Cryo-Protectant شامل انواع DMSO
تولید کننده انواع محلولهای ضد عفونی کننده و استریل زا برای لوازم و تجهیزات آزمایشگاهی و بیمارستانی شامل انواع انکیوباتورها ، کابینتهای کشت سلولی

NORDIC IMMUNOLOGY
هـــلند

بزرگترین تولید کننده آنتی بادی و معرفهای ایمونولوژی آزمایشگاههای تحقیقاتی ، پزشکی ، دامپزشکی

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ثبت شده در اداره کل تجهیزات پزشکی ایران
تلفن :۲۲۰۸۱۰۹۹ – ۲۲۰۸۱۱۱۳ فکس :۲۲۰۸۱۰۹۸ – ۲۲۰۸۱۱۱۴

Sinarvin Tajhiz

شرکت مهندسی

سینارووین تجهیز

نماینده انحصاری

شرکت بیوسیف در ایران

Sepax 2

-the essential choice-

نسل جدید تکنولوژی سیپکس

تکنولوژی سیپکس جهت فرآوری

سلولهای بنیادی از خون بند ناف و

مغز استخوان قابل استفاده در:

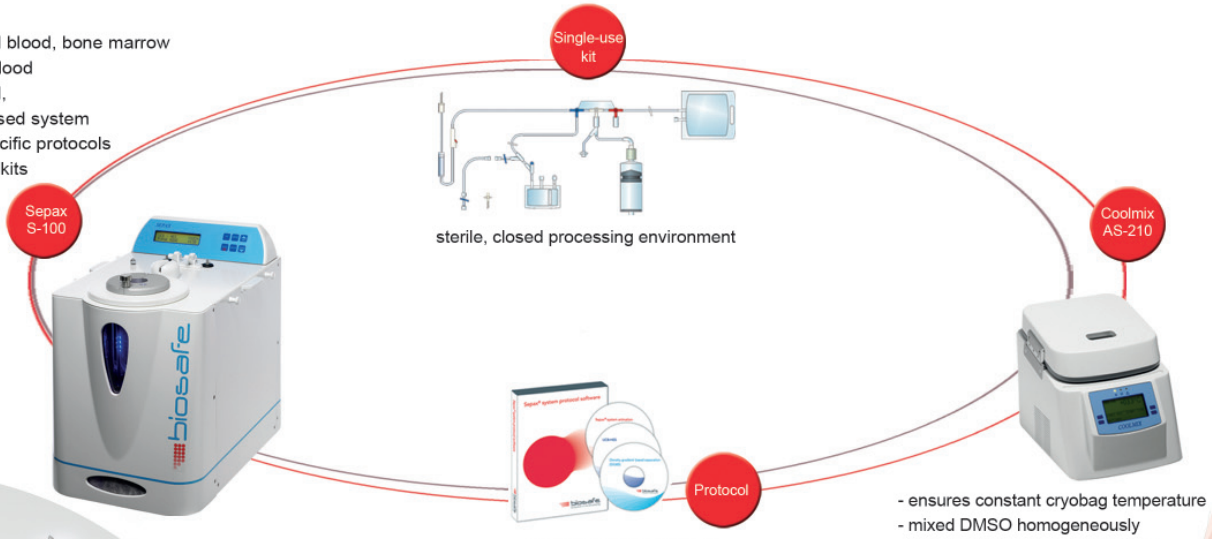
- بانکهای خون بند ناف
- مراکز سلول درمانی

تکنولوژی برتر

ساخت سوئیس

processed by Sepax

- processes cord blood, bone marrow and pripheral blood
- fully automated, functionally closed system
- application specific protocols and single-use kits
- fully mobile



- ensures constant cryobag temperature
- mixed DMSO homogeneously
- reproducible cooling and mixing

سابقه انجام بیش از ۶۰۰۰۰۰ پروسس

در ۷۰% اعضاء نت کورد

from benchtop to bedside

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World leader in adult stem cell processing

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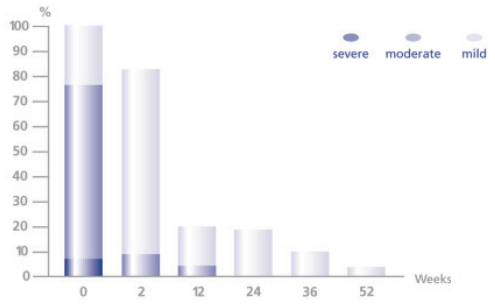
www.sepax.persianblog.ir

آدرس : مشهد - شهرک فناوری بیوتکنولوژی شمال شرق - واحد ۳۱۳

تلفن : ۰۵۱۱-۸۶۹۶۸۹۶ و ۰۵۱۱-۵۴۲۴۴۷۶ فاکس : ۰۵۱۱-۵۴۲۴۴۷۶

Effective symptom relief with Vagifem®.

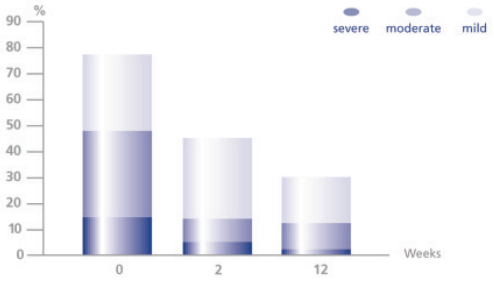
Vaginal dryness... after treatment with Vagifem®



More than 90% of the woman experienced a complete relief of vaginal dryness.

Mettler, *Maturitas 14, 1991*

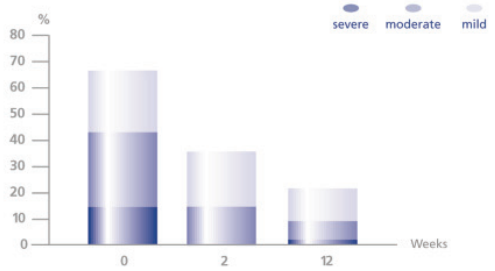
Itching, burning and soreness... after treatment with Vagifem®



After just 2 weeks more than 80 % had none or only mild symptoms.

Sindberg, *Eur J Obstet Gynecol Reprod Biol.1992*

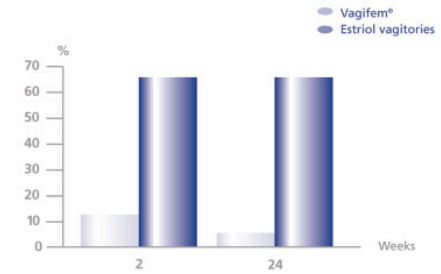
Painful intercourse... after treatment with Vagifem®



After 12 weeks almost 90% of the women had none or only mild symptoms of dyspareunia.

Sindberg, *Eur J Obstet Gynecol Reprod Biol.1992*

Leakage... minimised with Vagifem®



Vagifem® perceived as a clean, no mess delivery versus estriol vagitories.

R. Dugal et al., *Acta Obstet Gynecol Scand 2000, 79*

Vagifem®

25µg 17ß-estradiol

"Abbreviated Prescribing Information"

The following information is for your general knowledge. However, please refer to "Summary of Product Characteristics" (SPC) for more detailed information.

Vagifem® (film-coated vaginal tablets, containing estradiol)
Qualitative and quantitative composition: Each tablet core contains estradiol hemihydrate equivalent to 25µg of estradiol. Other ingredients are: Hypromellose, lactose monohydrate, maize starch, magnesium stearate, macrogol 6000. The vaginal tablets are marked "Novo 279". Each white tablet is contained in a disposable single-use applicator. There are 15 applicators with vaginal tablets in each box. **Labelled indication:** Treatment of atrophic vaginitis due to estrogen deficiency. The experience of treating women older than 65 years old is limited. **Contraindications:** Known , past or suspected breast cancer, known or suspected oestrogen-dependent malignant tumours (e.g. endometrial cancer), undiagnosed genital bleeding, untreated endometrial hyperplasia, previous idiopathic or current venous thrombo embolism (deep venous thrombosis, pulmonary embolism), known hypersensitivity to the active substances or to any of the excipients, porphyria. **Precautions:** Before initiating or reinstituting hormone therapy, a complete personal and family medical history should be taken. The following are conditions which need supervision, in case they are present, have occurred previously, and/or have been aggravated during pregnancy or previous hormone treatment: a history or risk factors for thromboembolic disorders, hypertension, diabetes mellitus, cholelithiasis, migraine headache, systemic lupus erythematous, history of endometrial hyperplasia, epilepsy, asthma. Treatment with this product has to be immediately withdrawn, in case of observing contraindicated conditions or any of the following conditions: jaundice or deterioration in liver function, significant increase in blood pressure, new onset of migraine-type headache, pregnancy. **Special warning:** Although the dose of estradiol in Vagifem® is low and the treatment is local, systemic absorption may occur to a minor degree, in some patients. **Pregnancy and lactation:** Vagifem® is not indicated during pregnancy. If pregnancy occurs during medication with Vagifem®, treatment should be withdrawn immediately. Vagifem® is not indicated during lactation. **Dosage and administration:** Vagifem® is administered deeply into the vagina, using the applicator. Initial dose is 1 vaginal tablet a day for two weeks. Maintenance dose is 1 vaginal tablet twice a week. Treatment may be started on any convenient day. **Adverse effects:** Common adverse events (>1/100, <1/10) are the following ones: genital candidiasis or vaginitis, headache, nausea, abdominal pain, abdominal distension or abdominal discomfort, dyspepsia, vomiting, flatulence, vaginal haemorrhage, breast oedema, breast enlargement, breast pain or breast tenderness, peripheral oedema, vaginal discharge or vaginal discomfort. **Drug interactions:** Due to topic administration of the low dose of estradiol in Vagifem®, interactions of clinical relevance are not expected.
Please refer to patient information leaflet for more information. **NOTE: "Vagifem® is a Prescription Only Medicine"**

Reference:
Vagifem® Patient Information Leaflet

MN-BKAV / HRT / 011 / Aug. 2010-01