the cells after 14 days. The constructs were observed under SEM 3, 7 and 14 day after seeding. Attachment of the fibroblasts to the surface of the scaffolds and their migration into the pores were demonstration on SEM. These results were also confirmed by histological analysis performed one week after cell seeding. Our results demonstrate that the gelatine/chitosan scaffolds fabricated with SLL method have a very high porosity and pore interconnectivity, and hence, are suitable for skin tissue engineering applications.

**Synthesis of Triangular Silver Nanoprisms: The Role of Reagents on Shape-Dependent Antibacterial Properties for Dental Applications**

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The osseointegration of dental implants is related to their composition and surface treatment. Osseointegration could be enhanced by incorporation of antibacterial agents like silver nanoparticles that reduce post-implantation infection. It has been shown that the antibacterial activity of silver nanoparticles could be enhanced by adjustment of their size and shape. In this work, a triangular silver nanoprism colloid was synthesized by chemical reduction of silver nitrate, using silver nitrate, trisodium citrate dehydrate, sodium borohydride, hydrogen peroxide and polyvinylpyrrolidone (PVP). The morphology and structure of the nanosilvers and effect of different amounts of reagents were investigated using UV-visible spectroscopy, X-ray diffraction and Transmission Electron Microscopy. The results demonstrated that higher concentrations of $NaBH_4$ prolongs the initiation time of nucleation. This shows that $NaBH_4$ serves as a capping agent, which may be derived from the adsorption of borohydride ions on nanoparticles, and stabilizes the silver nanoparticles. Also, citrate ions preferentially bound to the surface resulting in nanoparticles with plate structures. On the other hand, exclusion of PVP leads to shortening of initiation time of nucleation from ~25 to ~5 min. The advantage of using PVP is narrowing the size distribution. The antibacterial assay was performed by inhibition zone test against the gram-negative bacterium *Escherichia coli* and showed a higher antibacterial activity of triangular silver nanoprisms in comparison to conventional spherical nanosilver. It is anticipated that incorporation of the efficient antibacterial triangular silver nanoprisms into implant coating could enhance the osseointegration of dental implants after implantation surgery.

**Synthesis and Characterization of Gelatin/Nano β-TCP Composite Scaffold for Bone Tissue Engineering**

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The present study aimed at the evaluation of bioactivity, biodegradability and mechanical properties of gelatin/nano β-TCP bio-composite. The scaffold was fabricated by the salt-leaching method, cross-linking was performed using formaldehyde, and the microparticles of sodium chloride acted as the porogen agent. Characterization with XRD, SEM and FTIR showed that the sizes of the nanoparticles were about 100 nm and their shapes were spherical. On the other hand, the scaffolds showed a porous structure with pore sizes of 100-400 μm. SEM and EDX analysis of the nanocomposite scaffolds, seven days after immersion in the simulated body fluid (SBF), confirmed their bioactive properties. Meanwhile, biodegradability of the scaffolds were evaluated by immersion in SBF for different time periods, which demonstrated a reduction in the degradation rate of GTF group scaffolds due to the presence of β-TCP nanoparticles. Assessment of the mechanical properties of the scaffolds showed that the maximum compressive and bending properties occurred at 20wt% of β-TCP. The appropriate mechanical properties and biodegradation rate for tissue engineering applications obtained at scaffolds containing 1 gram of formaldehyde solution.

**A New Approach for Bone Therapeutics: Controlled Alendronate Delivery in 3D Porous Layered Double Hydroxides/Gelatin Scaffolds**

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Alendronate is a bisphosphonate drug, which is indicated for use in osteoporosis and cancer bone metastasis. As this medication has several side effects, it has been proposed that localized and controlled delivery of alendronate in a porous scaffold may decrease its side effects and allow its application for a wider range of indications. The objective of this work was to develop an alendronate-loaded controlled release nanocomposite in plasticized gelatin glue that would provide a suitable matrix to support proliferation and differentiation of osteoblasts. The alendronate anions were intercalated in calcium/aluminum layered double hydroxide (LDH) clay nanoparticles and dispersed in a 10% gelatin solution. This method has successfully controlled the release of alendronate by complete suppression of the burst phase. XRD, TTIR and SEM were used for structural study of the scaffold/carrier. The Drug release was measured with HPLC and FTIR. Also, the release process was assessed using a graded buffer with a range of different pH values. On the other hand, MTT assay, alkaline phosphatase measurement and cell staining were used to study the in vitro behavior of human primary osteoblasts. The results illustrated that LDH-based scaffolds can be used as appropriate carriers for delivery of alendronate.

**Developing Ex Vivo Expansion of Umbilical Cord Blood Hematopoietic Stem Cells to Produce Functional Cells for Transplantation**

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Umbilical cord blood (UCB) hematopoietic stem cells (HSCs) have been shown to be good alternatives for bone marrow (BM) hematopoietic stem cells, especially when a matched and related donor is not found for transplantation. But a great disadvantage of using cord blood hematopoietic stem cells is the low dose of these cells, which leads to slow immune reconstitution, and consequently, post-transplant infections and unsuccessful engraftment, particularly in adults and over-weight children. One of the promising approaches to overcome this drawback is *ex-vivo* expansion of hematopoietic immature stem cells, which increases the cell mass with less or no graft-versus-host disease. However, it should be noted that the routine methods of cell expansion have not improved the rate of engraftment as more mature progenitors, which are not the desired reconstituting cells, are produced by these methods. Naturally, HSCs reside in a cellular microenvironment composed of many signals, the interaction of which regulates hematopoiesis. This cellular microenvironment is mainly composed of cytokines, adhesion molecules and other molecules produced by mesenchymal stem/stromal cells (MSC), endothelial cells, fibroblasts, and certain blood cell types. It is suggested that wise genetic manipulation of these pathways would enhance programmed cell differentiation and ex vivo expansion of UCB stem cells and produce functional HSC required for transplantation. The results of recent studies magnify the need to improve methods of ex vivo expansion of HSCs, and therefore, keeping UCB transplantation the first and best option for more patients.

**The Effect of TGF-β3 on the In Vitro Chondrogenic Differentiation of Equine Adipose-Derived Mesenchymal Stem Cells**

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Regenerative medicine aims to replace or repair damaged organs and tissues including the injured joints and tendons. Research on the use of mesenchymal

stem cells (MSCs) in tissue regeneration has generally been driven by the needs of human and veterinary medicine. MSCs are one of the best candidates in treatment of musculoskeletal disorders. Nonetheless, the most suitable condition for their chondrogenic differentiation is under investigation. In this work, about 2-3 grams of equine fat tissue was isolated and transported to the laboratory. MSCs were isolated using mechanical and enzymatic digestion. Isolated cells were cultured in optimum medium until passage 3 (P3). To analyze chondrogenic differentiation, 500 000 P3 cells were cultured under tree different conditions: 1) basic medium as control, 2) differentiation medium without TGF-β3 and 3) differentiation medium supplemented with 10 ng/ml TGF-β3. Chondrogenic differentiation was applied for 21 days in pellet culture system and medium was changed every 3 or 4 days. Isolated MSCs were plastic-adherent, fibroblast-like and expressed specific markers. After the period of chondrogenic culture, no significant differences were observed in pellet sizes among 3 groups. Histological results confirmed chondrogenic differentiation in groups containing chondrogenic medium (2 and 3) compared with control group. More coherent extracellular matrix and typical lacuna formation confirmed better chondrogenic differentiation in the group supplemented with TGF-β3. The results suggest that addition of TGF-β3 to the standard chondrogenic medium is required for promotion of chondrogenic differentiation of equine adipose-derived MSCs.

**mRNA Expression of HOXB4 and GATA2 Self Renewal-Associated Genes in the Ex Vivo Expanded Cord Blood Hematopoietic Stem Cells**

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Ex vivo expansion of cord blood hematopoietic stem cells (CB-HSCs) has important applications in cell-based therapy. The ability of ex vivo expanded cells to maintain self renewal is essential for the widespread use of expanded cord blood cells in transplantation, gene therapy and regenerative medicine. HOXB4 and GATA2 are closely related to the self renewal potential of HSCs. Here, we expanded CB-HSCs in two different culture conditions and evaluated the mRNA expression of HOXB4 and GATA2 genes in ex vivo expanded cells. CB-HSCs were cultured in cytokine (SCF, TPO and FL) supplemented culture and in co-culture with mesenchymal stromal cells (MSCs). After 14 days of culture, the two culture conditions were compared in terms of expansion rate of TNC, $CD34^+$cells, $CD34^+/CD38^-$ cells and CFU-C count. Moreover, mRNA expression of HOXB4 and GATA2 genes in expanded $CD34^+$cells were analyzed by real time RT-PCR. Results showed that the expansion rate of TNC, $CD34^+$cells, $CD34^+/CD38^-$ cells and CFU-C was higher in co-culture condition compared to the cytokine supplementation. Also, the results indicated that in cytokine supplementation, the mRNA expression of HOXB4 and GATA2 genes was significantly decreased in the expanded $CD34^+$ cells. On the other hand, in co-culture condition, the decrease in mRNA expression of HOXB4 and GATA2 genes was negligible in expanded $CD34^+$ cells. These findings suggest that *ex vivo* expansion of CB-HSCs in the presence of MSCs increases the rate of proliferation and expansion of CB-HSCs, while retaining the mRNA expression of HOXB4 and GATA2 self renewal-associated genes in expanded cells.

**Effect of Strontium Substitution on the Microstructural and Biological Characteristics of 58S Bioactive Glasses**

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The biological benefits of strontium in bone growth and repair have been shown by several studies, which warrant local delivery of this ion by slow release from specially designed bioactive materials. As the ionic radius of strontium (1.16 Å) is close to that of calcium (0.94 Å), it can be integrated into bioactive materials by substitution for calcium in many crystalline lattices. In this study, strontium was substituted for calcium in a series of sol-gel derived 58S bioactive glasses. Thermal analysis (TGA/DTA) was used to determine the thermal behavior and stabilization temperature of the samples. The samples were soaked in a simulated body fluid (SBF) and the formation of hydroxyapatite was investigated. In addition, osteoblastic cells were cultured in a medium containing the extracts of different samples. The results showed that further addition of strontium to the glass structure promoted the crystallization before and after thermal stabilization. Also, it was noted that strontium release from the glass matrix significantly accelerated the hydroxyapatite formation after soaking in SBF. Furthermore, in the glasses that contained higher amounts of strontium, strontium apatite ($Sr_{10}(PO_4)_6OH$) was detected as a new phase. In addition, there was no significant difference in term of toxicity between the glasses containing different amounts of strontium. It has been concluded that the substitution of strontium for calcium in 58S bioactive glass materials could enhance apatite formation in biological environments which is an indication for bone bonding of bioactive materials. The cell culture studies approved that all the samples were biocompatible.

**Kinetics of Tissue Growth In 3D Polymer-Based Nanocomposite Scaffolds: Effect of Particle Size on Cell Proliferation and Differentiation**

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Nanotopography is a highly important factor that affects cell-substrate interactions, and hence, should be considered for functional design of the materials aimed to promote tissue regeneration. Here, an indirect 3D printing method was used to fabricate polymeric scaffolds with controlled architecture and interconnected porous structure with macro- (400–500 μm) and micro- (~25 μm) porosities. Polycaprolactone (PCL) was used as a model system to study the kinetics of tissue growth. The surface of the scaffold was decorated with topographic patterns using nano and micro scale bioactive glass particles. Due to better match to the nanoarchitecture of ECM, this study aimed to evaluate the effects of particle size on the cellular response of mouse preosteoblastic cells. Tissue growth and enzymatic activity on 2D films and 3D scaffolds showed that the nanotopographical features, material stiffness, and surface curvature affect the cellular adhesion, proliferation, and kinetics of tissue growth into the MC3T3-E1 seeded pore channels. It was demonstrated that the nanoscale topography and higher stiffness of the substrate derived from nanoparticles impaired cellular adhesion and proliferation in 3D structures. Evaluation of alkaline phosphatase activity showed that hard particles influence the local bone nodule formation and thus, the differentiation of the cells on 2D films. The results of this study implies that the existing knowledge of the effects of nanofeatures (e.g. nanotubes, nanogrooves, nanopost, nanopit arrays) on 2D structures and films may not be applicable for 3D scaffolds, which are utilized to support cell proliferation and differentiation in non-load-bearing areas for enhancement of bone regeneration.

**Dental Materials with Improved Mechanical Properties – A Possibility to Modify Conventional Calcium Hydroxide Cements**

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The conventional calcium hydroxide cements are minimally invasive dental restorative materials. Their relatively poor mechanical properties impose some limitations on their applications. On the other hand, hydroxyapatite (HAp) nanoparticles exhibit excellent properties as ideal candidates for dental applications. These nanoparticles demonstrate enhanced densification due to higher surface area, which can improve the mechanical properties of the composites. To enhance the performance of dental cements, it is possible to add HAp nanoparticles, that resemble the crystals found in the tooth enamel, into their structures. In this research, HAp nanoparticles have been prepared via a wet