

chemical precipitation method from the aqueous medium containing calcium hydroxide and orthophosphoric acid. The HAP nanoparticles were added to calcium hydroxide dental cements, which are widely used as pulp capping materials. The newly formed cements were characterized by X-ray diffraction, scanning electron microscopy, and X-ray energy dispersive analysis. The results showed that adding 5 wt.% HAP nanoparticles could improve the mechanical strength and increase the calcium release rate as a mineralization promoter without deteriorating the antibacterial behavior and setting time of the cement (which was in an acceptable clinical range). These improved characteristics of conventional calcium hydroxide cements found to be critical for clinical translation as a new type of dental cements. In addition, the protection efficiency of the improved cements against the penetration of fluids, bacteria and bacterial toxins, as the ultimate goal of this study, is under evaluation.

#### **The Effects of Cell Density in Primary Culture on Isolation of Bone Marrow-Derived Mesenchymal Stem Cells**

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Mesenchymal stem cells (MSCs) are multipotent cells used in clinical trials for tissue regeneration, especially for treatment of locomotor disorders. Bone marrow (BM) is a typical source for isolation of MSCs. However, the available volume of bone marrow and the number of its cells are limited. In this study, we aimed to determine the effects of the cell density of the primary culture on the success of isolation of MSCs from bone marrow aspirates. For this purpose, the bone marrow samples aspirated from two horses underwent density centrifugation. Then, the buffy coat of mononuclear cells was collected, washed and cultured with different densities for 21 days as passage 0 (P0). At day 14, the number of colonies that had more than 15 cells was determined. Also, at the end of P0, the confluency was estimated and the total cell number was determined. The results showed that minimum percent of confluency, colony numbers and total cell number were obtained from samples with minimal density ( $1 \times 10^5$ ) and maximum numbers of colonies, total cell number and confluency belonged to the samples with the maximal cell density in primary culture ( $1 \times 10^9/\text{cm}^2$ ). In conclusion, the number of the isolated mesenchymal stem cells is directly related to the density of the cells in primary culture i.e. a higher density yields a higher number of cells with a higher percentage of confluency. Nonetheless, even minimal cell number in primary culture ( $1 \times 10^7/\text{cm}^2$ ) gave enough number of MSCs which can be used for either clinical applications or basic studies.

#### **Isolation and Characterization of Horse Bone Marrow Mesenchymal Stem Cells for Treatment of Joint Injuries: An Animal Model for Human Studies**

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Osteoarthritis is the most common human and animal joint diseases encountered world-wide. General strategies for restoration of the damaged joint surfaces are (1) enhancement of the intrinsic healing capacity of cartilage, (2) replacement of the damaged cartilage, and (3) regeneration of the articular cartilage surface. Animal models are widely used to develop and evaluate the reconstruction of articular cartilage. Due to the similarities in size, load, and type of injuries of joints, horse is the most appropriate animal for modeling joint diseases of humans. As the first step in regenerative strategy is isolation and characterization of competent stem cells, bone marrow was aspirated from 3 mares. Samples were mixed with culture medium and were centrifuged using high density liquid. Mononuclear cells in buffy coat were collected, washed and cultured. After 3 passages (P3), putative mesenchymal stem cells (MSCs) were analyzed for morphological, gene expression, differentiation and growth characteristics. The cells were slender and spindle-shaped, and expressed common surface markers for MSCs including CD29, CD44, CD 90, and MHC-I, but not CD34 (a hematopoietic stem cell marker) and MHC-II. P3 cells differentiated into adipocytes, chondrocytes and osteocytes when they were cultured in appropriate differentiation medium. Also, the isolated cells were able to form colonies. Based on these results, we succeed to isolate and characterize horse MSCs from bone marrow. According to the chondrogenic differentiation potential of these cells, they can be used in regenerative medicine for treatment of joint injuries in horse, as the best animal model for human joint diseases.

#### **Can calcium Hydroxide have a Positive Effect on Dental Cements?**

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In order to prolong the release of drugs in dental cements, their microstructural characteristics could be changed. However, this is a critical process that might have a negative effect on the mechanical properties of the final set cement. For example, the presence of porosity in zinc polycarboxylate (ZPC) cements affects their physico-chemical characteristics in a number of different ways. In the current study, different concentrations of calcium hydroxide have been added to ZPC dental cements to investigate its positive effects on the microstructural properties of the cements. The powder and liquid phases of the cement were mixed (23°C and humidity of around 50%). The samples were prepared by placing the freshly mixed cements in cylindrical molds (12 × 6 mm in height and diameter). The results showed that addition of calcium hydroxide to the ZPC cement matrix clearly affected the microstructural properties (porosities and their size distributions). By adding small amounts of calcium hydroxide, around 5 wt.%, the setting time increased but remained in a clinically acceptable range. In contrast to our expectation, changing the porosity of samples enhanced the mechanical strength, which could be attributed to the newly formed chemical bonds. From these results, it could be concluded that adding calcium hydroxide to the cement matrix had a meaningful effect on the characteristics of the set cements. Therefore, in order to design effective dental cements with drug delivery capability, it may be appropriate to change the porosities by adding calcium hydroxide to their structure.