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Bone regeneration with a combination of nanocrystalline hydroxyapatite silica gel, platelet-rich growth factor, and mesenchymal stem cells: a histologic study in rabbit calvaria

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Objective. This study aimed to assess NanoBone as a carrier construct for mesenchymal stem cells (MSCs) and platelet-rich growth factor (PRGF).

Study Design. In the calvarial bone of 8 mature New Zealand White male rabbits, four 8-mm defects were created. Each defect received one of the following treatments: Group 1, 0.2 mg Nano-hydroxyapatite (HA) granule + 2 mL culture medium; Group 2, 0.2 mg Nano-HA + 1 mL autologous PRGF + 2 mL acellular culture medium; Group 3, 0.2 mg Nano-HA + 2 mL culture medium containing 100,000 autogenous MSCs; Group 4, 0.2 mg Nano-HA + 2 mL culture medium containing 100,000 autogenous MSCs + 1 mL autologous PRGF.

Result. Histomorphometric analysis at 6 and 12 weeks demonstrated significantly higher bone formation in group 4 (29.45% and 44.55%, respectively) ($P < .05$). Bone formation in groups 1, 2, and 3 were as follows: 11.35% and 32.53%, 29.10% and 39.74%, and 25.82% and 39.11%, respectively.

Conclusions. NanoBone with MSCs and PRGF seems to be an effective combination for bone regeneration in a rabbit calvaria model. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:e7-e15)

Nanoscaffolds as the new "state of the art" in the science of delivery systems are carrier constructs made in form of a grid, a scaffold, or a reticulum of nanofibers, and prepared in a multilayer structure. For each layer, fiber characteristics, thickness, and diameter can be separately controlled, which in turn allows for the manipulation of the porosity, surface area, and thickness of the nanoscaffold.[1-3] NanoBone (Artoss, Rostock, Germany) is a granular composite of synthetic nanocrystalline hydroxyapatite (HA) and silica gel matrix, consisting of interconnected pores sized 10 to 20 nm.[1] The large surface area of NanoBone (84 m^2/g) is attributable to its high porosity (60%), which provides a scaffold with high osteoconductive and angiogenic properties.[2,4] Favorable biocompatibility and biodegradability of the product, make NanoBone a potential replacement for autografts in the future of bone-augmentation procedures.[2-5]

The controlled absorbency and high porous structure of nanoscaffolds might also affect the proper adherence of mesenchymal stem cells (MSCs) to the carrier and their further differentiation.[6] Although the proven proliferative and osteogenic differentiation capabilities of multipotential MSCs[7-9] has rendered them major associates in bone-regeneration operations,[10,11] MSC delivery with synthetic or natural scaffolds has shown a limited amount of bone formation in comparison with autogenous bone graft.[12] To overcome this shortcoming, several studies have mainly focused on the amelioration of cell carriers,[13-15] whereas others have tested the inductivity of various growth and differentiating factors on MSC-based osteogenesis.[16-18]

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Plasma-rich growth factor (PRGF) and platelet-rich plasma (PRP) are autologous constituents of inductive factors obtained from blood.[19,20] These compositions have high concentrations of platelets and contain different active ingredients, such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), and fibrin products[21,22]; when used with particles of bone substitutes, they produce an easily handled mixture that has been reported to be effective in bone augmentation procedures.[19,20] The concomitant application of PRP with MSCs and a scaffold has shown favorable bone regeneration in animal models.[2,23,24] The results, however, are not yet comparable to autologous bone graft in human models, calling for further research to perfect the available combinations.

Combined use of nanoscaffolds with growth factors and MSCs is a complex investigation with low examined evidence. Following the proven osteoconductive (as well as osteoinductive) capacities of NanoBone,[25] the present study mainly aimed to test the potential of this scaffold as a carrier construct for stem cells and growth factors; and to histologically evaluate the effects of simultaneous application of NanoBone, PRGF, and MSCs on bone regeneration in a rabbit calvarial defect model.

MATERIAL AND METHODS

Animals

Eight mature New Zealand White male rabbits (mean weight 2.5 kg) were used in this study. Rabbits were kept in separate cages, in a room at ambient temperature, and were fed a standard commercial rabbit chow. Water was provided ad libitum. All animal procedures were approved by the Ethical Committee for Experimental Animal Research, according to the guidelines of Tehran University (Tehran, Islamic Republic of Iran). In the calvarial bone of every animal, four 8-mm defects were created. Each defect received one of the following treatments: Group 1, 0.2 mg Nano-HA granule + 2 mL acellular culture medium; Group 2, 0.2 mg Nano-HA with 1 mL autologous PRGF and 2 mL acellular culture medium; Group 3, 0.2 mg Nano-HA + 2 mL culture medium containing 100,000 autogenous MSCs; and Group 4, 0.2 mg Nano-HA with 2 mL culture medium containing 100,000 autogenous MSCs and 1 mL autologous PRGF.

Isolation and cultivation of MSCs

From the femur of each animal, 5 mL of bone marrow was aspirated and was transferred into heparinized tubes. The aspirate was diluted at 1:3 in Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK). On day 1, nonadherent cells were discarded. Adherent cells were washed with phosphate-buffered saline (PBS; Gibco), and were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin. Third passage cells, suspended at the concentration of 1×10^6 cells/mL, were used for the following experiments (Figure 1).

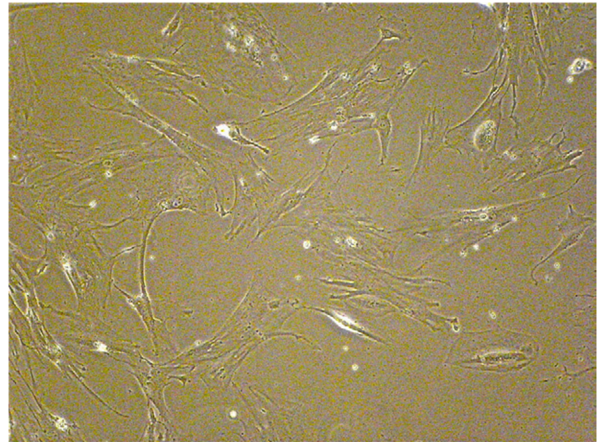

Fig. 1. Third-passage cells under light microscope (original magnification ×400).

Flowcytometry analysis

Fluorescence absorbance cell sorting analysis was performed using standard protocols and quantification criteria. The gate to distinguish positive cells from negative cells was set individually for each marker. Fluorescent isothiocyanate (FITC)-conjugated monoclonal antibodies against the following markers were applied: CD34 (Mittenyi Biotech, Bergisch-Gladbach, Germany), CD13, CD105, CD29, and CD44 (BD Bioscience, San Diego, CA). Antibodies were used at 2 mg/mL concentration at 4°C for 30 minutes. FITC-labeled mouse immunoglobulin G painted cells were set as the negative control group. Cells were washed twice with PBS and fixed with 1% paraformaldehyde. Each specimen with more than 99% fluorescent-labeled cells of the whole cellular population was considered as positive.

In vitro osteogenic differentiation

To identify the isolated cells as MSCs, their differentiation potential into osteogenic cell lineage was also evaluated. Cells from the third passage were provided with an osteogenic culture medium containing DMEM, 50 μg/mL ascorbic 2-phosphate (Sigma Aldrich, St. Louis, MO), 10 nmol/L dexamethasone (Sigma), and 10 mmol/L β-glycerol phosphate (Sigma). After 3 weeks, differentiated cells were fixed for 1 hour with 4% formalin and rinsed with PBS (Gibco). Mineralization of extracellular matrix was visualized after staining

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