Noncollagenous Matrix Proteins in Bone Healing After Local Delivery of Controlled-Release Simvastatin/Poly Lactic Glycolic Acid Microspheres

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Statement of Purpose: Numerous strategies for bone regeneration have been carried out in the last years, and drug delivery technologies by using bioresorbable polymers are a promising strategy. Simvastatin (SIM), 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, used clinically for reduction of cholesterol synthesis to prevent cardiovascular disease, has recently been proven to enhance the healing of critical-sized bone defects. It stimulates BMP-2 gene expression in osteoblasts, as well as osteopontin, and alkaline phosphatase in osteoblastic cells and bone marrow-derived cells. [1, 2]. Bone regeneration can be optimized by the association of SIM with PLGA [3] which allows local and controlled drug delivery, avoiding its metabolism in the liver when systemically administered. In the present study, we evaluated the potential bone healing effect of 2.5% SIM loaded onto a PLGA in ratio of 50/50 bioresorbable scaffold, applied locally into critical-size defects in rat parietal bone.

Methods: Bone defects 5mm in diameter were created in the right parietal bone of sixty 3-months-old male rats. After this procedure, 20 received PLGA membrane (M), 20 had the defect filled with PLGA+SIM microspheres and covered with PLGA membrane (MSI), and 20 rats healed spontaneously (C). After 4 or 8 weeks, the rats were euthanized and their calvariae were fixed and processed for light (LM), transmission (TEM) and scanning (SEM) electron microscopy. Some samples were embedded in LR White acrylic resin, and the 80-nm thick sections were incubated with polyclonal OPN antibody followed by protein A-colloidal gold (15 nm) conjugate, before counterstaining with uranyl acetate and lead citrate to immunocytochemistry analyses. Some paraffin sections were incubated for osteopontin (OPN), osteoadherin (OSAD) and bone sialoprotein (BSP).

Results The examination of the bone defect margins at LM revealed that the neoformed bone in the MSI group at both time points presented a mature pattern with few regions of immature bone, which was confirmed by the typical immunolabeling for OPN and BSP. These proteins were more intensely immunolabeled in M and C groups, which presented predominance of primary bone trabeculae into the defects. OSAD was localized in newly deposited matrix surrounding the osteoblasts in all groups. TEM analysis revealed that MSI group presented neoformed bone arranged facing the microspheres area, which were adhered to fibroblasts interspersed with abundant collagen fibrils that were regularly packed exhibiting a lamellar bone aspect. Ultrastructural immunocytochemistry at day 30 of MSI specimens revealed OPN-rich cement lines at wound margins and into the primary bone.

Conclusions: The presence of OPN regulates the establishment of a cement line, the earliest mineralization event in the bone healing process. The results offer evidences that the addition of a single topical application of simvastatin associated with PLGA microspheres can accelerate the regeneration of bone defects possibly by the stimulation of proliferation and differentiation of mesenchymal cells into the defect area and improves the quality of the new bone. It indicates that SIM/PLGA facilitates bone regeneration and should be proposed as an osteoinductive agent to treat areas in which bone healing involves cellular components as osteoblasts, stem cells and growth factors.

References:

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