COMBINED EFFECT OF BMP-2 GENE THERAPY AND BIOACTIVE GLASS MICROSPHERES IN ENHANCEMENT OF NEW BONE FORMATION

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Introduction
Bioactive glasses (BG) are a group of surface-active silica based synthetic biomaterials. BG surface favors osteoblastic proliferation and BG also forms direct chemical bonding with ongrowing new bone. Chemical bonding is based on firm attachment of the new bone apatite with the apatite reaction layer of BG. Biologic activity can be regulated by varying the chemical composition of BGs. Adenovirus-mediated recombinant human BMP-2 (RAdBMP-2) gene transfer has been found to have significant osteoinductive potential at both orthotopic and ectopic sites (1,2,3). The present study examined the effect of RAdBMP-2 gene therapy combined with bioactive glass microspheres in promotion of new bone formation. Our hypothesis was that bioactive glass surface could provide optimal osteoconductive conditions for cellular action of osteoinductive RAdBMP-2 gene transfer.

Material and methods
Harlan Dawley female rats (n=72) (age 15-19 weeks, weight 205-334g) were used. Each animal underwent unilateral surgery of right or left tibia in a random order. The study protocol was approved by the Institutional Animal Care Committee. Using standard surgical techniques and continuous saline irrigation, a round cortical window (Ø 2.8 mm) was drilled into the anteromedial cortex of the proximal metaphysis of the tibia. For lavage of the medullary cavity, a smaller unicortical hole (Ø 1.0 mm) was drilled 5 mm distally. Bone marrow was exhaustedly removed by rinsing with 0.9 % saline. The medullary space between the cortical holes was filled with BG microspheres. Bioactive glass microspheres (Ø 250-315 µm) were manufactured (Abmin Technologies Ltd.) using a spraying method. The composition of the selected glass (glass 13-93) was SiO2 53 %, Na2O 6 %, CaO 20 %, K2O 12 %, MgO 5 %, P2O5 4 % by weight. Adenoviral vectors RAdBMP-2 carrying the BMP-2 gene or RAdLacZ (kindly provided by Dr. Seppo Yla-Herttuala) harbouring the Escherichia coli LacZ reporter gene under the control of cytomegalovirus (CMV) promoter mixed with 0.9 % saline were injected locally (10^5 plaque-forming units [pfu]) into the medullary spaces filled with BG microspheres. The control defects were filled with BG microspheres only and sham-injected with saline. Empty control defects were also used and left to heal without any filling after saline rinsing. The rats were killed 4 days, 2 weeks, and 8 weeks after surgery and the tibias were harvested. The bones were scanned with a pQCT device (Norland Stratec) and the mean density value (mg/cm^2) of the defect area was divided by the mean density of the two adjacent cortices and expressed as a percentage. After pQCT scanning, the specimens were subjected to high-resolution radiography. For the histomorphometric analyses, 20 µm sections were prepared in the horizontal plane at the site of the larger cortical window and stained with a modified van Gieson method. The amount of new bone formation was measured by using a computerized image analysis system (Micro-Scale TC) and expressed as a percentage of the cross-sectional medullary area.

The relative area occupied by bioactive glass microspheres was also measured in order to follow the time-dependent resorption of the biomaterial. Each of the four groups (BG/RAdBMP-2 group, BG/ RAdLacZ group, BG only group and empty control) had 6 animals at each time point for the analyses. The results of the measurements were expressed as the means ± standard deviations of means and the statistical comparison of the four groups were based on the one-way analyses of variance with a post hoc t-test.

Results
Based on pQCT imaging and histomorphometry, the cortical bone window was anatomic, with constant location and of equal size. The defects showed uniform filling with BG microspheres (Fig. 1). The amount of BG microspheres decreased from 43.6 ± 7.8 % at four days to 20.0 ± 5.8 % by eight weeks. There were no significant differences between the groups in the resorption of BG microspheres. The pQCT density decreased on an average of 27.5 % by eight weeks and there were no significant differences between the BG-filled groups. The pQCT density of non-filled control defects remained significantly lower than of the BG-filled defects.

In histology, there was moderate formation of immature woven new bone (primary bone response) in all defects at two weeks (Fig. 2), with the highest value of 19.7 ± 5.1 % in control defects left to heal without filling. This was significantly (p=0.016) higher than in defects filled with combination of BG/RadLacZ. At eight weeks, there was significantly more new bone in defects treated with BG and RAdBMP-2 than in defects left to heal without filling (p=0.003) (Fig. 2). New bone formation in defects filled with BG only or BG/RadLacZ was also higher than in control defects, but the differences did not reach a statistical significance (p=0.10 and p=0.06, respectively). Reflecting a different healing pattern, BG-filled defects showed a time-related increase of defect new bone, whereas in control defects the amount of intramedullary new bone started to decrease significantly already between two and eight weeks (p=0.013). The same difference was observed in the closure of the cortical bone window. Complete or near-complete cortical bone bridging was observed virtually in all control defects, but only in 61% (11 out of 18) of BG-filled defects with or without a RAdBMP-2 or RAdLacZ adjunct.

Discussion
The current study showed that local BMP-2 gene therapy enhances new bone formation on bioactive glass microspheres. The current defect model provided ideal conditions for testing the direct action of RAdBMP-2 gene transfer because of the natural presence of mesenchymal stem cells in the medullary cavity. In many clinical situations, the bone healing conditions are less ideal with a need of stem cell transplantation. In those situations, the RAdBMP-2 gene could be transferred to mesenchymal stem cells ex vivo before combined application with bioactive osteoconductive biomaterial.

It is likely that the induction of new bone in the medullary cavity in BG-filled defects changed the strain environment of the cortical window to an extent that the tubular bone lost the natural biomechanical signal for early closure of the cortical window. In control bones, new bone formation was only transient in the medullary cavity and this type of healing was associated with early closure of the cortical bone window.

References