Therapeutic Gene Targeting To Endogenous Stem Cells For Bone Regeneration

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Disclosures:

Introduction: Massive bone defects constitute a complex medical condition. These injuries not only cause great suffering to patients, but also lead to long-term hospitalization and immobilization, repeated surgeries, loss of working days, and considerable costs to the health system. Bone grafts are the main treatment for these defects. In the US alone about 1.5 million bone grafts are implanted annually generating $2.5 billion sales. It is well known that autologous bone grafts (autografts) are considered the gold-standard therapy. Yet these grafts are not always available, and their harvest often leads to prolonged postoperative pain and comorbidity at the donor site. Structural allografts are readily available from tissue banks and, therefore, are an alternative to autografts. Unfortunately, allografts have very low osteogenic potential, leading to poor graft-host integration that results in numerous failures due to fractures and nonunion. Autografts are potent bone inducers probably because they contain osteogenic cells, growth factors, and an osteoinductive extracellular matrix. Several studies attempted to mimic this process of bone formation by combining osteoprogenitor cells (mainly mesenchymal stem cells [MSCs]), growth factors (mostly BMPs), and scaffolds made of biomaterials. It was shown that MSCs engineered to overexpress different BMP genes can differentiate and contribute to the process of bone formation in vivo, leading to complete regeneration of bone defects. However, such an approach requires several steps_cell isolation, expansion, and engineering_which complicates and prolongs the regulatory pathway to clinical use. An alternative targeted gene therapy approach would be to overexpress an osteogenic gene, such as a BMP, in MSCs residing at the defect site. This is an attractive approach because it results in a transient secretion of the BMP protein at physiological levels and it does not require harvesting of cells or grafts, or the production of high-cost reagents such as recombinant proteins. Yet the efficiency of gene expression strongly correlates with the vector carrying the gene to the target site. A wide array of gene delivery methods have been developed and tested for bone regeneration over the years. There is no doubt that viral vectors are the most efficient gene delivery tools, but their efficiency is offset by potential risks of tumorigenic and immunogenic reactions. Nonviral vectors are considered safer for human use, albeit much less efficient for gene expression. To enhance the efficiency of in vivo nonviral gene delivery, methods that rely on a short pulse of energy were developed. These methods induce the formation of transient nano-sized pores in the membranes of cells, enabling the uptake of DNA, which leads to cell transfection. Cell poration can be induced by an electric pulse, ultrasound, or laser energy. Indeed, previous attempts have shown the feasibility of inducing bone formation using in vivo cell poration and direct delivery of plasmids encoding for a BMP gene. However, none of these studies were performed in a site of bone injury. We hypothesized that attraction of endogenous MSCs to a bone defect site, using a collagen-based scaffold, followed by ultrasound-based BMP gene delivery would induce efficient bone regeneration.

Methods: We evaluated the use of ultrasound-mediated gene delivery for bone defect repair in two rodent models. A 2.5 mm defect was created in the radius bone of C3H/HeN mice and a 2X2 mm cylindrical defect was created in the coccygeal vertebrae of Wistar rats. Next we implanted a collagen scaffold (Duragen Integra LifeScience), allowed cell migration into the scaffold for 10 days, and then injected the 50 ugr of plasmids encoding for BMP-2, -6 or Luciferase (as a control) into the scaffold, under fluorescent guidance, followed by a short pulse of ultrasound using a Branson Digital Sonifier 450 (20KHz, 13 mm probe, at 22.7W/cm2 for 1-minute x2. Target site was cooled by submerging in water). In order to demonstrate host endogenous stem cell migration into the collagen scaffold, animals were sacrificed 7 days post gene delivery and the treated bones were subjected to immunohistochemistry using antibodies against: Luciferase, CD29, 90 and 44. Reporter gene expression was monitored non-invasively using Bioluminescent Imaging (BLI). Bone regeneration was analyzed using microCT imaging (uCT 40, Scanco) 3.5-5 weeks post gene delivery. In addition, we performed a pilot study in a minipig where we implanted a collagen scaffold in a 1cm tibia segmental defect. Ten days later we injected 1 mg of GFP plasmid, suspended in 10% microbubbles (Optison, GE Healthcare) to the defect followed by an ultrasonic pulse (using Mettler Electronics Sonicator 740X at 1MHz, 2.2 W/cm2, Dusty Cycle: 50% for 10 minutes). The procedure was repeated on the next day. The day after, we sacrificed the pig and evaluated gene expression using an optical imaging system (IVIS, Caliper). Finally we used flow cytometry to characterize the cells that populated the collagen scaffold implanted in the pig’s tibia.

Results: Our results in the rodent studies showed that the cells that migrated into the collagen scaffold implanted in the site of bone defects were stained positively to the MSC surface markers CD29, 90 and 44. The same cells also expressed the Luciferase...
transgene. Flow cytometry analysis of the cells isolated for the collagen scaffold implanted in the pig tibia indicated that 90-95% of the GFP-positive cells (i.e. the cells that were transfected by the transgene) were also positive for CD 29 and 90. BLI showed that the Luciferase reporter gene was detectable for only for 24-35 days in the rodent models. MicroCT analysis showed that significantly more bone volume was generated in rodent bone defects when BMP-2 or -6 plasmids were overexpressed at the defect site. Interestingly, BMP-6 delivery resulted in more gap bridging in the radius defect model compared to BMP-2 (Figure 1). Finally, bone regeneration was limited to the defect site and we did not observe any overgrowth.

Discussion: In conclusion, we have shown that transient BMP gene expression in endogenous stem cells is sufficient to induce significant bone regeneration in rodent models of long bone and vertebral injuries. Moreover, we have shown the feasibility of the system in a pig model of tibia segmental bone defect. To the best of our knowledge, we are the first to demonstrate that bone regeneration can be achieved using endogenous stem cell recruitment and a transient, ultrasound-mediated, gene delivery.

Significance: The described therapeutic approach could be highly attractive for orthopedic surgery since it does not require any bone grafting.
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Figure 1: Targeted BMP-2 and -6 ultrasound-mediated gene delivery induces radius bone defect repair in a mouse model. Segmental bone defects were generated in mouse radii and implanted with collagen scaffolds. Ten days later, 50 μg BMP-2, -6 or Luc plasmids were injected into the defect, and then a short pulse of sonoporation was applied using a sonicator. 3.5 weeks after sonoporation, the bones were imaged using a micro-CT scanner. Results indicated that defect regeneration was achieved and that new bone formation was localized to the site of the original defect. In the control groups, treated with the reporter Luc, only minimal bone regeneration was observed.