BONE FORMATION STIMULATED BY TREATMENT WITH HEALOSMP52 IN A SHEEP MODEL OF AVN

INTRODUCTION
With varying and uncertain etiologies, avascular necrosis (AVN) of the femoral head is a degenerative disease that usually leads to hip joint destruction. The pathology of osteonecrosis is initiated by an ischemic event resulting in bone and marrow necrosis, inflammation, infarction of the lesion by fibrous scar tissue, stress fractures in the weakened subchondral bone, and collapse of the articular surface of the femoral head. To obviate end-stage arthropathy, current treatment methods to preserve the femoral head include electrical stimulation to induce osteogenesis, core decompression to relieve marrow hyperpression, osteotomy to remove necrotic bony tissue, and bone grafting to strengthen the subchondral bone and facilitate healing. It has been proposed that the limited success of these treatments could be improved by the use of bone grafts that incorporate growth and differentiation factors to stimulate bone regeneration [1].

HealosMP52 is a mineralized collagen bone grafting material, Healos®, containing growth/differentiation factor-5 (GDF-5), a member of the bone morphogenetic protein family [2]. New bone formation induced by HealosMP52 has been demonstrated in various animal models, wherein GDF-5 acts as the osteoinductive stimulus within the osteoconductive Healos® scaffold [3,4,5]. The purpose of this study was to evaluate the ability of HealosMP52 to promote bone formation in a sheep model of avascular necrosis. Necrotic lesions simulating AVN were created in the femoral head and neck, and treated with HealosMP52. Treated defects were evaluated by gross observation, microradiography, and histological methods for bone formation at 6 and 12 weeks after treatment, in comparison to untreated lesions.

METHODS

Implant Material: Under aseptic conditions, Healos®, an osteoconductive mineralized collagen bone grafting matrix, was saturated with a solution of recombinant human GDF-5 (rhGDF-5, Biopharm GmbH, Heidelberg, Germany) and lyophilized. The resulting HealosMP52 contained 0.5mg GDF-5/cc of Healos® matrix.

Surgical Procedure: Nineteen skeletally mature (> 4 years old), female, Columbian X Rambouillet sheep (60 to 80kg) were used, 5 animals used to pilot the surgical technique and 14 animals for the HealosMP52 study. All procedures using animals received IACUC approval (Protocol #0-094-01). Similar to a procedure previously described [6], defects were created in the left proximal femur by drilling with an 8mm drill bit to a depth of 3.5cm using a lateral approach to the greater trochanter up into the femoral neck and head. Necrosis was produced by treating the hole with 1mL of 70% ethanol for two minutes followed by a saline flush. The cortical end of the hole was sealed with sterile bone wax, soft tissues were routinely closed, and animals were allowed to recover. After 6 weeks, defects were re-exposed, bone wax was removed, scar tissue was curetted out, and each lesion was filled with a 5cc strip of HealosMP52 (total 2.5mg GDF-5) or left untreated. Animals were labeled with calcein at 7 days after treatment and with tetracycline at 16 days prior to euthanasia at 6 weeks (n = 3) or 12 weeks (n = 4). At necropsy, defects were assessed by gross observation and lesions were evaluated by microradiographic and histological methods.

Microradiography and Histology: Contact microradiographs were taken of whole, excised proximal femora. Slab sections were taken through the center of the lesion and contact microradiographs were taken of the sections to obtain the best sample. Slabs were processed for embedding, embedded in Technovit 7200 (Kulzer), sectioned to 200µm thickness, and ground to 50 to 70µm (EXAKT system). Two sections were made for each specimen, one stained with toluidine blue and one unstained for fluorochrome labeling assessment as an indication of new bone formation.

RESULTS
At surgery, accurate placement of the drill into the femoral neck was challenging. However, the five animals used to pilot the surgical procedure allowed practice of the surgical approach and verification that the lesion could be located for treatment during the second surgery after 6 weeks. The surgeries well tolerated, and animals recovered their normal gait within a few days following each surgical procedure. Six weeks after drilling, defects were filled with soft tissue, indicating that the ethanol was effective in producing local tissue necrosis. At 6 weeks after treatment, histological evaluation of toluidine blue stained sections showed active intramembranous bone formation and lamellar remodeling was in progress in lesions treated with HealosMP52. In untreated defects at this time point, a modest amount of bone formation was observed primarily at the periphery of the defect and within the surrounding bone. At 12 weeks after treatment, the promotion of healing and bone regeneration by implants of HealosMP52 was clearly evident (Figure 1). Three of 4 treated specimens showed a majority of the defect space to be occupied by newly formed, sponge-like woven bone with thickened trabecular bone present in some areas. Residual implant material could not be discerned in these treated defects. In contrast, untreated defects generally contained large cysts, granulation tissue, hematomas centrally and minimal new bone formation peripherally. One of 4 untreated defects showed partial repair, near the defect opening at the cortical end. Similarly, fluorochrome labeling was markedly more pronounced in defects treated with HealosMP52 in comparison to untreated defects. The diffuse and discrete pattern of labeling indicated ongoing remodeling and maturation of the reparative bone in defects treated with HealosMP52.

DISCUSSION
The necrotic, non-healing defects created in this sheep model appear to mimic the pathology of AVN in humans. The results of this preliminary study show that treatment of these defects with HealosMP52 stimulated bone regeneration after 12 weeks, with ongoing bone formation and remodeling observed. Thus, HealosMP52 could serve as an inductive bone graft material to promote formation, remodeling and strengthening of the subchondral bone, and potentially avert the crippling consequences of AVN.

References:

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