Characterization of a Chronic Tibial Defect Model in Goats

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Disclosures:

Introduction: Current large animal models do not accurately recapitulate the complexity and severity of nonunion fractures and military extremity trauma, or allow differentiation among the efficacy of grafting materials. We recently developed a caprine chronic tibial defect (CCTD) model that incorporates loss of regional bone, periosteum and soft tissue, local tissue scarring, and fibrous membrane formation by using a polymethylmethacrylate (PMMA) spacer to evaluate new bone tissue engineering/regeneration options (1). There was minimal regenerate bone within these defects three months after engraftment with fresh autogenous cancellous bone (ACB) when local muscle loss and PMMA spacer with a smooth surface were included. The current study used this model to compare defect healing three months after engraftment with five current, commonly used grafting strategies.

Methods: During the initial surgery, 9 cm of periosteum, 5 cm of tibial diaphysis, and 10 cm3 of muscle were removed, a bone cement spacer placed in the defect, and the tibia stabilized with an IM locking nail in skeletally mature female goats (~ 65 kg BW). Four weeks later, the PMMA spacer was removed and the defect encompassed by the induced membrane was filled with: 1) 10-12 cm3 of fresh ACB graft from the sternum, 2) 10 cm3 of morsellized cancellous allograft (MCA) alone, 3) MCA + 10 cc sterile bone marrow aspirate (BMA), 4) MCA + BMA + rhBMP-2 (2 mg on absorbable collagen sponge), or 5) MCA + rhBMP-2 with 6 goats/group. Orthogonal radiographs taken every 4 weeks assessed defect healing. MicroCT analysis and histology of the regenerate tissue were performed 12 weeks after grafting after euthanasia.

Results: Five goats were euthanized prior to the end of the study; 2 from the MCA + BMA + BMP group, and 1 each from the ACB, MCA, and MCA + BMP groups. The remainder of the goats had good limb use after each surgery. The cancellous bone and marrow collected from the sternum were rich in hematopoietic elements (Fig. 1) with significantly more progenitor cells compared to that from the proximal humerus and iliac crest. After 12 weeks of healing following engraftment, the greatest amount of new bone formation was seen radiographically in the defects treated with MCA + BMA + BMP with bone bridging the entire defect (Fig 2A). The poorest healing was seen in the defects treated with MCA and MCA + BMA (Fig 2C,D) and the amount of new bone in the ACBG defects was intermediate relative to the other treatment groups (Fig 2B). Micro CT analysis of the defects confirmed the results of the radiographic data and predict the greatest resistance to load in the MCA + BMA + BMP treated defects (Fig. 3).

Discussion: We further validated our chronic caprine defect model, demonstrating differences in the degree of bone healing in defects treated with grafting strategies used in current clinical practice. Bone healing appears to be compromised relative to large animal models of acute critical sized defects that heal completely after treating with the clinical “gold standard” graft material, fresh autogenous cancellous bone (2-5). We were able to distinguish differences in new bone formation among the various grafting methods in this study. The greatest degree of healing was seen when osteogenic, osteoinductive and osteoconductive materials were all delivered to the defect site, whereas graft material that provided osteoconductive material alone resulted in minimal new bone formation. This model more closely represents the type of wound present in chronic non-union fractures and extremity injuries of military personnel that refuse to heal despite multiple grafting procedures.

Significance: We have developed a chronic bone defect model in the goat tibia that incorporates loss of regional soft tissue and periosteum, local tissue scarring, and fibrous tissue induced by the use of a local defect spacer to differentiate among grafting strategies. This model will be useful for the next wave of advancement in the clinical science of bone regeneration and the systematic assessment and optimization of new technologies or integrated strategies.

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References: 1. Pluhar et al. 2013 ORS poster #1539.
Figure 1. Histology section of a sternal body demonstrating highly cellular cancellous bone with hematopoietic elements (H&E staining).
Figure 2. Radiographs taken 12 weeks after grafting with A) MCA+BMA+BMP, B) ACBG, C) MCA, or D) MCA+BMA showing differences in bone healing.
Figure 3. Plots created from microCT data demonstrating the effect of ACBG (left panels) vs. MCA+BMA+BMP (right panels) on % bone.