Current definitive treatment of large diaphyseal tibial bone defects includes cancellous autograft, Ilizarov distraction osteogenesis, and vascularized bone grafting. These methods are associated with significant complications and donor site morbidity. Bone graft substitutes, specifically biodegradable composite scaffolds, offer promise for treating these defects while negating donor site morbidity. A biodegradable composite scaffold consisting of poly-D,L-lactide-co-glycolide acid (PLGA) and calcium phosphate has shown promise as a bone graft substitute both in vitro and in vivo. Calcium phosphate (CaP) is employed both as a surface coating and an internal particulate within the scaffold. The scaffold contains interconnecting macropores ranging from 500 to 1500µm to allow for efficient host tissue invasion.

The aim of our study was to evaluate bone formation and angiogenesis produced within a biodegradable PLGA/CaP scaffold when used to treat a diaphyseal tibia defect and compare this to an iliac crest autograft, the gold standard for treatment, or leaving the defect empty.

Methods

Permission for the use of 18 mixed breed canines was obtained from the St. Michael's Hospital Animal Care and Ethics Committee (Toronto, ON, Canada). An 8.0mm diaphyseal tibia defect was created in a canine model. All tibiae were reamed to 7.0mm and fixed with a 6.5mm statically locked intramedullary nail. Eighteen canines were allotted to one of three treatment groups: 1) left empty (N=5), 2) treated with iliac crest autograft (N=6), or 3) treated with a PLGA/CaP biodegradable scaffold (Tissue Regeneration Therapeutics Inc., ON, Canada) (N=7).

Fluorescent markers were given at successive time periods: calcein green at 6 weeks, xylol orange at 9 weeks, and tetracycline at 11 and 14 weeks. Animals were sacrificed at 15 weeks and their legs were perfused with a barium compound. Samples were analyzed using plain radiography, Micro CT, and brightfield and fluorescent microscopy.

All statistical calculations were carried out using SPSS Windows Version 14.0 (SPSS Inc., IL, USA). A one-way analysis of variance (one-way ANOVA) was used to compare means of the three treatment groups. Significance was reported for p < 0.05. Post-hoc analysis was performed using Tukey’s HSD. A two-tailed test was used with significance reported for p<0.05.

Results

Bone growth around the defect site in empty and scaffold samples was markedly different than the autograft samples. Radiographs from samples in the scaffold and empty groups showed bone formation from the proximal and distal osteotomy sites that grew into the defect site over time. Bone growth in the autograft samples proceeded from the autograft itself with limited bone formation at osteotomy sites.

Micro CT and brightfield images of scaffold samples displayed multiple blood vessels surrounding and within the substance of the scaffold (Fig.1A and 1B). These vessels ranged in size from 10 to 100µm and were filled with barium compound. This amount of angiogenesis was not observed in the empty or autograft samples. Although bone growth did not occur within the scaffold, there was significant bone formation at the osteotomy site adjacent to the scaffold within the 9 to 14 week period (Fig. 1C). This bone formation may be the result of angiogenesis occurring within the scaffold.

The volume of bone within the tibial defect site was reported as a percentage of the total volume of the defect site. The mean percent bone volume within the defect site was not significantly different between treatment groups (p=0.112). The volume of blood vessels within the tibial defect site was reported as a percentage of the total volume of the defect site. There was a significantly greater percent blood vessel volume within the defect site of the scaffold group as compared to the autograft group (p<0.001).

Bone formation at the osteotomy sites was defined as the distance from the original osteotomy site to the tip of newly formed bone measured from brightfield images. Bone formation at the osteotomy sites was significantly greater in the scaffold group than the autograft group (p=0.015). There was no significant difference between the scaffold and empty groups (p=0.697), or between the empty and autograft groups (p=0.163). Osteotomy sites associated with greater angiogenesis displayed greater bone formation.

Bone formation rates were reported as the distance between the fluorescent bone labels for each of the 3 time periods: 6 to 9 weeks, 9 to 11 weeks, and 11 to 14 weeks. These measurements were recorded at 4 anatomic sites around the tibial defect: the endostem, the cortex, the periosteum and the osteotomy site. Autograft samples had the greatest bone formation rates within the periosteum for all three time periods. Autograft and scaffold samples had the greatest rate of bone formation within the cortex for all three time periods. There was no significant difference in bone formation rates between treatment groups within the endostem or at the osteotomy site for any of the 3 time periods.

Discussion

Our canine tibial defect model provides a satisfactory facsimile of the traumatic tibia fracture with associated bone loss. Our results suggest that both angiogenesis and osteogenesis around an osteotomy site may be improved with delayed bone grafting. We have also shown that bone formation at an osteotomy site in the tibia increases with improved angiogenesis. The PLGA/CaP biodegradable scaffold we have employed promotes angiogenesis within a bony defect and could be used in conjunction with autografting to avoid either two-stage or delayed operative procedures.

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