Introduction

An intra-operative technique of collecting and concentrating the buffy coat from a unit of blood has previously been characterized. This autologous approach may be an alternative to other potential osteoinductive therapies such as bone morphogenetic proteins, or conceptually may be used as a synergistic carrier for BMPs or osteoprogenitor cells. Platelets contain a number of growth factors, including Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor Beta (TGF-β), Fibroblast Growth Factor (FGF), Insulin-like Growth Factor (IGF), and Vascular Endothelial Growth Factor (VEGF), which have been demonstrated to have an active role during various stages of the bone healing cascade. These factors have been shown to enhance vascular tissue ingrowth, increase the migration of osteoblasts and osteoprogenitor cells to the local site, cause these cells to multiply in number, and influence their differentiation down the osteoblast lineage progression. Termed Autologous Growth Factors (AGF™), this ultracentrifugated platelet solution also contains fibrinogen at three times greater than native levels, and will form a reliable, firm gel upon the addition of a small volume of thrombin. This pilot study evaluated the addition of AGF Gel to a bone graft substitute in a segmental defect model using the canine radius.

Methods

AGF Gel Implant Preparation: One unit (450ml) of blood was collected at the time of surgery from each animal. The blood was run through an Electromedics Elmd-500 cell washer to separate the buffy coat (platelets and white cells). A centrifuge speed of 5600 rpm was used to fill the bowl with red cells and draw off the platelet poor plasma. The bowl was slowed to 2400 rpm and influence their differentiation down the osteoblast lineage progression. Termed Autologous Growth Factors (AGF™), this ultracentrifugated platelet solution also contains fibrinogen at three times greater than native levels, and will form a reliable, firm gel upon the addition of a small volume of thrombin. This pilot study evaluated the addition of AGF Gel to a bone graft substitute in a segmental defect model using the canine radius.

Surgical Model: Bilateral 30mm critical sized segmental defects were created in the radii of eight mongrel dogs. One defect was grafted with Pro Osteon 500R granules plus AGF Gel, while the other received an equal amount of Pro Osteon 500R alone as a paired control. Following a medial approach, a 2.7mm 10-500R granules were simultaneously applied and mixed with the bone graft, then allowed to gel for 30 seconds before extrusion from the syringe. The platelet concentrations in the blood, BC, and AGF concentrate are shown in Table 1. Samples were taken from the blood, BC, and AGF for platelet counts on a Coulter AcT-10. To create the composite implant, 10cc of Pro Osteon 500R granules plus AGF Gel, while the other received an equal amount of Pro Osteon 500R in the blood to 636 x10^3 platelets/µl in the BC, a 3.1-fold increase. Processing into AGF yielded 1,494 x10^3 platelets/µl, a 7.3-times overall increase.

Discussion

Pro Osteon 500R is an osteoconductive bone graft substitute with an interconnected porosity consisting of hydroxyapatite and calcium carbonate, and has been shown to predictably resorb over 6-18 months. The polymer sheet used to keep the loose granules from migrating also served to prevent soft tissue interposition. Its macroporous nature also has been shown to facilitate bone regeneration through vascular ingrowth and mesenchymal cell migration.

The exclusion of three of the eight animals combined with the variability of the results suggest that in general the plates used were not adequate to support full weight bearing in this canine segmental defect model. The accompanying motion resulting from the loose hardware would also help account for the relatively low bone volume observed in the defect sites. Despite the variability, the addition of AGF to the implant did result in an increase in the amount of new bone formed. While additional studies are needed to verify these results, this pilot study suggests that the use of autologous concentrated platelets as a source for multiple growth factors to enhance bone regeneration may be a viable alternative to recombinant therapies.


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