The Addition of Autologous PRP Improved the Bone Healing Response in Two Bone Graft Materials when Compared to the Addition of Bone Marrow Clot in a Canine Multi Femoral Defect Animal Model

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Introduction
The importance of the bone marrow (BM) clot environment in connective tissue progenitor (CTP) transplantation and the possibility that this environment might be replaced or even improved using platelet-rich plasma (PRP) presents the need and opportunity to understand and optimize factors that contribute to clot related efficacy. Platelets contain growth factors which promote and accelerate bone healing. PRP, which concentrates platelets and also reduces red blood cell and unselected marrow-derived nucleated cells is expected to increase bone formation. Also, within the graft site, the addition of PRP would contribute to less necrotic debris, less inflammation and a “more mature” (i.e. more lamellar vs. woven) bone. Data is presented for the addition of autologous PRP and bone marrow clot on two different bone scaffold materials enriched with osteoprogenitor cells using a method of Selective Retention (SR), in a Canine Femoral Defect Model.

Materials and Methods
Study design: Comparisons between BM clot and PRP clot were performed in two sets of experiments, 10 animals per experiment. Experiment (1) bone graft material used Polycaprolactone (PCL/TCP) cylindrical 3 D printed scaffolds and Experiment (2) bone graft consisted of canine bone allotransplants comprised of demineralized long bones and mineralized cancellous chips, approximately 3mm diameter, provided by LifeNet (Virginia Beach, VA). Prior to the start of surgery, heparinized bone marrow aspirates were obtained from each humerus. The method of Selective Retention (SR) was used to concentrate and select bone marrow progenitor cells onto the graft material.

PRP clot preparation: Prior to surgery, 50 ml of autologous blood, was obtained and centrifuged following EXACTECH® centrifuge, (Gainesville, FL) instructions to obtain a platelet rich plasma (PRP) sample. Thrombin (Topical, 5,000 IU and 10% CaCl₂) were combined and added to PRP.

Bone Marrow clot preparation: At the time of scaffold implantation, 2 ml of non-heparinized bone marrow were aspirated and added to SR prepared scaffolds and allowed to clot.

Surgical procedure: Skeletally mature male coonhounds were used (mean body weight 33 kg). Four identical 10mm diameter 15mm deep unicortical cylindrical defects for grafting were created in the left femur. Scaffolds enriched with progenitor cells plus the addition of either PRP or bone marrow clot was distributed among the four defect locations. PRP and BM clot preparations were assigned and scaffolds were implanted following an ABAB or BAAB pattern. Due to a therapeutic focus on rapid bone formation, animals were euthanized at 4 weeks.

Micro CT analysis: Quantitative micro CT analysis of each defect site was used to define the distribution of new bone formation, and in-growth into each defect site. These data were visualized in radially oriented two dimensional contour plots based on Percent Bone Volume (%BV). Bone formation in the pericortical (PC) region and in the intramedullary (IM) region were plotted and analyzed separately, with the expectation that scaffolds perform differently in these two different biological environments.

Histological Assessment: Specimens were fixed overnight in 10% neutral buffered formalin, dehydrated, and embedded in methylmethacrylate plastic without decalcification. After the plastic has polymerized, the blocks were sectioned at approximately 5 µm using a motorized microtome. The resulting sections were stained with goldners trichrome, and hematoxylin and eosin (H&E).

Results
Micro CT analysis: The %BV 2D contour plots in Figure 1 illustrate that new bone formation was more extended with the addition of clotted PRP for both materials. In the case of the allograft, greater %BV was detected in the center-most radial region of the defect site while a trend towards lower %BV was observed in the peripheral regions. More advanced bone remodeling toward native bone and bone marrow occurred in the intramedullary region. Figure 2 shows that overall there is a moderate but not statistically significant increase of the %BV with the use of PRP clot compared to the use of BM clot for bone substitutes.

Histological Evaluation: For Allograft with bone marrow clot or PRP clot, bone in-growth was found extended in the pericortical region. Allograft with PRP clot showed very robust bone in-growth, with the area of new formation extended outside of the pericortical region. In some areas, woven bone was covered with thick seams of osteoid, which were covered by osteoblasts. Cellular activity with osteoblasts and osteoclasts occurred on the surface of the mineralized bone (new bone), and not within the demineralized allograft (Fig 4). The histology slides confirmed that remodeling of the intramedullary canal was at an advanced stage with a good remodeling of the allograft matrix and high vascularization.

Discussion
These results demonstrate that PRP moderately accelerated bone maturation when added to osteoprogenitor-enriched scaffolds, particularly in the deeper/central (less vascularized) areas of the defect. The results support the theory and expectation that PRP would perform better compared to a marrow clot, because it would result in a comparable growth factor environment within the defect, while also limiting the number of red blood cells (RBCs) transplanted into the defect as non-contributing cells.

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Reference