A NanoComposite Scaffold for Bone Tissue Engineering

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INTRODUCTION: Over one million reconstructive surgery, trauma, or abnormal skeletal defect operations are performed in the United States annually. To achieve reconstructive goals, large amounts of autologous or alternative large bulk allograft are needed in the surgical procedure. Allograft bone material and alternatives such as synthetic grafts lack the necessary cellular and other biological components for bony union and healing to occur. We have recently developed a composite scaffold which can be utilized for bone tissue engineering strategies [1,2]. The scaffold consists of nano- to micron-sized fibers having a uniform dispersion of nanoceramics embedded in the fibers for improved cellular attachment, infiltration and bone bioactivity. The nanoceramics are composed of 20/80 (wt.%/wt.%) hydroxyapatite/beta-tricalcium phosphate (HA/TCP) ceramic, which is a ceramic composition identified to accelerate bone marrow derived stem cell induced bone formation both *in vitro* and *in vivo*.[3] 20/80 HA/TCP has been shown to be more favorable in directing stem cell induced bone formation over HA or TCP alone. The Young's modulus of the composite scaffold is similar to trabecular bone, yet has an ultimate tensile strain of approximately 30% [1], demonstrating its mechanical flexibility for inserting into a defect site. This study evaluated the composite scaffold for the repair of large bone defects in both small and large animal models. We evaluated two formulations, a fast degrading composite prepared with polylactic glyocolic acid (PLGA) or a slow degrading composite prepared with polycaprolactone (PCL). The composites were also combined with whole bone marrow to mimic the clinical scenario where bone marrow can be harvested from the patient at the time of surgery and loaded onto the scaffold as an intra-operative point-of-care technology.

METHODS: All surgical procedures were approved by the Rutgers University Institutional Animal Care and Use Committee. Composite scaffolds were prepared either using PLGA or PCL with 30 wt.% nanoceramics (PLGA COMP or PCL COMP, respectively). Scaffolds without nanoceramics served as controls (CTRL). Scaffolds had an average interfiber spacing /pore size of 261$\pm$31 μm and porosity of 79.2$\pm$1.6%. Scaffolds with or without bone marrow (BM) were evaluated in 5 mm segmental bone defects in femurs of male BB Wistar rats (250-300g) for up to 8 weeks. This defect size results in a non-union if left untreated, as shown previously [4]. Scaffolds with or without BM also were evaluated in 10 mm x 18 mm bilateral corticocancellous bone defects in the distal femur of male, adult wether Suffolk sheep (64-101 kg.) at 8 and 16 weeks post-implantation. Histology, histomorphometry and microCT analyses were performed for both rat and sheep studies. One-way and Two-way ANOVAs and planned comparisons using Tukey-Kramer were conducted to determine statistical significance at $p<0.05$.

RESULTS: **Rat Segmental Defect Study**: In rat segmental defects by 8 weeks, complete bridging of the defect occurred for the PLGA COMP+BM (Figure 1A). By histomorphometry, higher %bone filled the defect for PLGA COMP as compared to PLGA CTRL(without ceramic) (n=5 per group, $p<0.05$). **Sheep Femoral Defect Study**: In the sheep studies, microCT (Figure 1B & C) and histomorpmetry demonstrated that significantly higher bone volume fraction (BVF) occurred for PLGA COMP+BM in comparison to PLGA COMP alone by 16 weeks. For the slower-degrading composite prepared with PCL, significantly higher BVF occurred for PCL COMP and PCL COMP+BM as early as 8 weeks in comparison to PCL CTRL (without ceramic). Significantly higher BVF for PCL COMP+BM as compared to PCL CTRL was determined at 16 weeks. Histomorphometry demonstrated approximately 80% of the PLGA COMP and 60% of PCL COMP degraded by 16 weeks. Sample size for sheep studies were n=4 to 5 per group per time point.

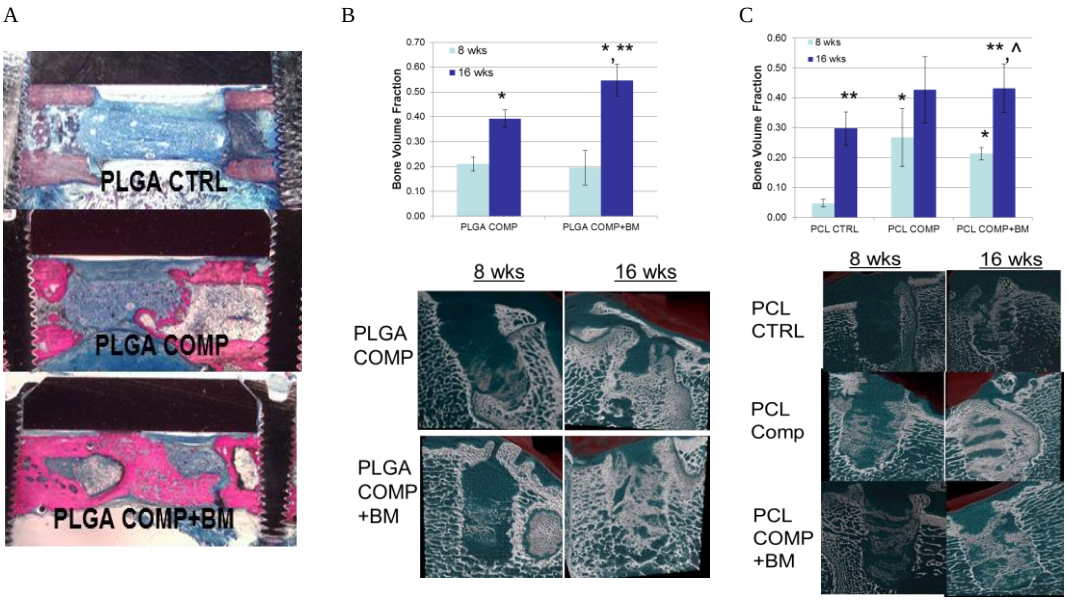

Figure 1: (A) Histology in rat segmental defects at 8 weeks post-implantation for PLGA CTRL (without ceramic), PLGA COMP, and PLGA COMP+BM, MicroCT analysis (bone volume fraction) at 16 weeks post-implantation in sheep defects for (B) PLGA COMP and PLGA COMP+BM , *$p<0.05$ between 8 and 16 wks and **$p<0.05$ between PLGA COMP+BM and PLGA COMP and (C) PCL CTRL (without ceramic), PCL COMP, and PCL COMP+BM, *$p<0.05$ higher than PCL CTRL, **$p<0.05$ between 8 and 16 wks and ^$p<0.05$ between PCLCOMP+BM and PCL CTRL.

DISCUSSION: Bone repair occurred in defects treated with the composite scaffolds. Both rat and sheep studies demonstrated that the use of nanoceramics in the composites enhanced repair over the use of polymer alone. The addition of bone marrow also accelerated repair with a more pronounced effect occurring for the fast-degrading PLGA composite group. Findings demonstrate the feasibility of the composite scaffolds for bone defect repair.
SIGNIFICANCE: Treating large bone defects remain a clinical challenge. Findings from this study demonstrate the potential of a composite scaffold for use alone or in combination with bone marrow cells for bone repair.
REFERENCES: 1. Patlolla et al, *Acta Biomat.*, 2010, 2. Patlolla et al, *Biotech. Bioeng.*, 2014, 3. Arinzeh et al, *Biomaterials*, 2005, 4. Brietbart et al, *J. Orthop. Res*, 2010.
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