Guiding hydroxyapatite scaffold based bone regeneration *in vivo* with collagen membranes

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INTRODUCTION: While autologous bone grafts from the iliac crest remain the gold standard for filling bone defects, there is a growing demand for using synthetic bone substitutes to overcome donor site morbidity and supply, especially for large segmental defects.[1] Scaffold parameters to be addressed included a) perfusion, b) materials selection to promote tissue integration, c) architecture that facilitates tissue penetration, d) mechanical strength and stability, and e) defects greater than 3 mm in depth. With hydroxyapatite (HA) being known for its osteoconductive property,[2] highly porous HA scaffolds address these issues. Furthermore, membranes have been used to successfully guide tissue regeneration in maxillofacial bony reconstruction.[3] The synergistic effect of HA scaffolds with guided collagen membranes to regenerate bone tissues *in vivo* was compared to autologous bone grafts in this study.

METHODS: Using a template coating process,[4] HA scaffolds with high porosity (85%) and interconnectivity were prepared with an average pore size of 340 µm. Using an approved protocol by the IACUC at the US Army Institute of Surgical Research, a 10 mm defect was created in the rabbit radius mid diaphyseal, as shown in Fig 1a and 1b.

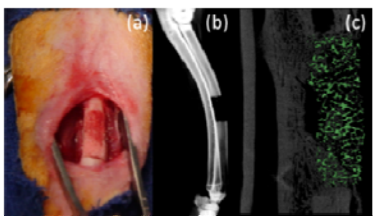

Figure 1. (a) Scaffold placement in rabbit radius defect and (b) radiograph showing defect in diaphysis. (c) Micro CT image showing scaffold (green) and bone in-growth in grayscale after 8 weeks surgery.

The defect was then either a) implanted with HA scaffold (8 animals), b) implanted with HA scaffold followed by a collagen wrap (scaffolds + wrap) (7 animals), c) implanted with autologous bone grafts (8 animals) as positive controls), or d) left untreated (8 animals) as negative controls. Collagen wraps ("Cytoplast RTM", Ossten Inc.), hydrated with normal saline, were placed around the scaffold implanted defects, extended adjacent to the ulna and secured with sutures. No other form of fixation was used for the animals implanted with scaffolds or autologous bone grafts. At 8 weeks after surgery, bony in-growth was examined using microCT (Fig 1c). Mechanical functionality was measured *ex vivo* by a 4-point flexural test, and contralateral limbs of animals receiving each treatments were used as controls. The bone mineral density (BMD) of the contralateral limbs for all groups was measured by ashing a 4 mm section of the mid diaphysis of the forearm. The section was weighed and volume measured by helium pycnometry to calculate BMD.

RESULTS: When comparing to animals that received no treatments, Fig 2a showed significantly higher flexural strength for animals with defect limbs implanted with either scaffolds + wrap, or autologous bone grafts when compared to no treatment controls. Similar trends and increased flexural toughness was also observed for the animals receiving different treatments when compared to their contralateral limbs (Fig 2b). The BMD of contralateral limbs for animals implanted with scaffolds and scaffolds + wrap were significantly greater than animals implanted with autologous bone grafts, with contralateral limbs for animals receiving no treatments having the lowest BMD among the four groups tested (Fig 2c). Compared to defect limbs implanted with scaffolds, micro-CT showed significantly greater bone in-growth in defect limbs implanted with scaffold + wrap at 8 weeks after surgery (Fig 2d).

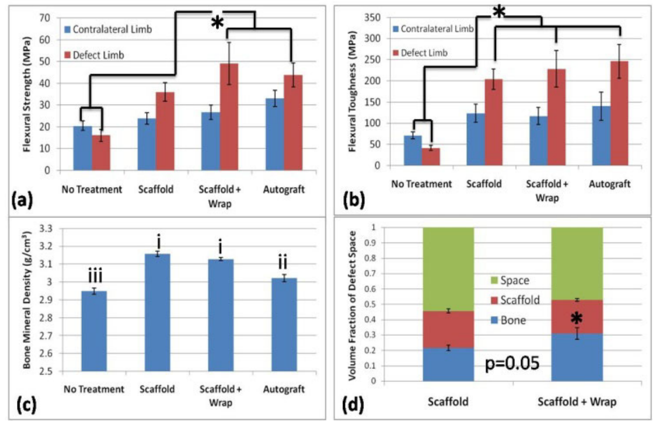

Figure 2. Graphs showing a) flexural strength, b) flexural toughness, c) BMD of contralateral limbs, and d) new bone formation for animals implanted with scaffolds and scaffolds + wrap after 8 seeks surgery. Statistical differences are grouped.

DISCUSSION: The use of a collagen wrap was observed to improve bone in-growth in porous HA scaffolds. With mechanical properties (strength and toughness) of defect limbs implanted with scaffolds or scaffolds + wrap being comparable to defect limbs implanted with autologous bone graft, these treatments were observed to have significantly higher mechanical properties over defect limbs with no treatments at 8 weeks surgery. Bone has the dual function of maintaining mechanical integrity as well as maintaining calcium homeostasis. The BMD of contralateral limbs of animals receiving either scaffolds, scaffolds + wrap, or autologous bone grafts were significantly higher than the contralateral limbs of animals receiving no treatments, suggesting that the implantation of HA scaffolds in defect limbs may assist in the preservation of BMD in the skeleton.

CONCLUSIONS: Flexural strength and toughness of defect limbs implanted with scaffolds or scaffolds + wrap were concluded to be comparable to defect limbs implanted with autologous bone grafts, with these treatments assisting in the preservation of BMD in the contralateral limbs of the animals. It was also concluded from this study that defects implanted with scaffold + wrap resulted in significantly greater bone in-growth when compared to scaffold alone at 8 weeks after surgery.

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