Acellular Collagen-Hydroxyapatite Scaffolds Support Osteoinduction After Subcutaneous Ectopic Implantation

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Statement of Purpose: Current synthetic bone graft substitutes provide suitable osteoconductivity but are not sufficiently osteogenic or mechanically robust for surgical handling, fixation, and load-bearing [1]. We have developed biologically-inspired collagen-hydroxyapatite (Col-HA) scaffolds with a tailored architecture and order of magnitude improvements in mechanical properties compared to conventional freeze-dried scaffolds, providing a microenvironment designed to support osteogenesis [2]. Therefore, the objective of this study was to evaluate the osteogenicity of these scaffolds in a subcutaneous ectopic murine model.

Methods: Col-HA scaffolds were prepared from a mixture of concentrated collagen fibrils (~180 mg/mL), paraffin microspheres (~375 μm), and HA whiskers, which was compression molded at 1 MPa, dried, leached of paraffin, crosslinked in 20 mM EDC and 8 mM NHS in 80% ethanol, rinsed, and rehydrated in PBS [2]. Scaffolds in this study were prepared with 85% porosity and 0, 20, or 40 vol% HA (n = 8/group). Scaffolds were implanted in ectopic subcutaneous pockets in the cervical dorsal region of eight, 4 week old female athymic nude mice [3] for 6 and 12 weeks. Following fixation, explanted scaffolds were imaged via micro-CT (μCT-80, Scanco) to quantify the volume of new bone formation (*BV*). Explants were embedded, sectioned, stained by H&E and labeled by immunohistochemistry for osteocalcin (OC) and osteopontin (OP).

Results: The scaffold mechanical properties were well-suited for surgical handling and resisting contraction during implantation, due to exhibiting a compressive modulus at least one order of magnitude greater than comparable freeze-dried scaffolds and fully recoverable elastic deformation upon repeated loading. *De novo* bone formation was evidenced by a measured increase in *BV* for all col-HA scaffolds, but not for collagen scaffolds (Fig. 1). All scaffold explants exhibited complete cellular infiltration and significant matrix deposition after 6 weeks implantation (Fig. 2). After 6 weeks implantation, col-HA scaffolds exhibited dense cell populations and matrix deposition, vascularization, positive staining for OC, and positive staining for OP, which increased by 12 weeks (Fig. 2). Collagen scaffolds exhibited no evidence of OC or OP. These results suggest that the scaffold architecture was conducive to the infiltration of endogenous cell populations and HA induced the differentiation of these cells into osteoblasts, likely via adsorption of endogenous proteins. Positive OC staining indicated the presence of osteoblasts and mineralization. Positive OP staining indicated the presence of osteoblasts and suggested the beginning of remodeling. Thus, acellular col-HA scaffolds were demonstrated to induce angiogenesis and osteogenesis after subcutaneous ectopic implantation, suggesting the scaffolds were osteoinductive.

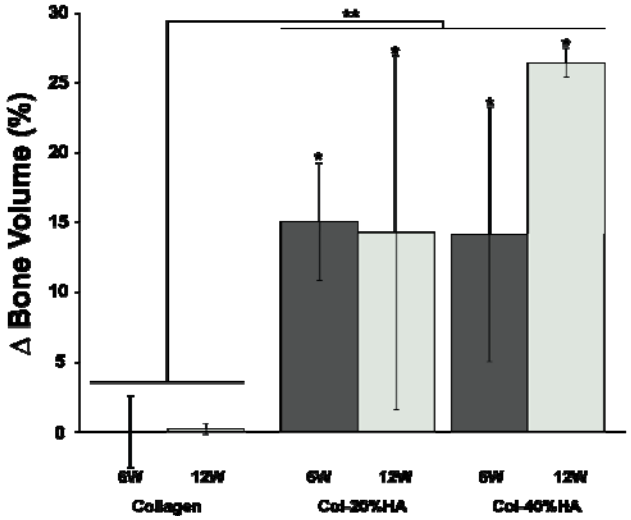

Figure 1. *De novo* bone formation in ectopically implanted col-HA scaffolds was evidenced by increased BV measured by micro-CT. *$p < 0.05$ vs. zero, **$p < 0.05$ vs. collagen scaffolds, Wilcoxon.

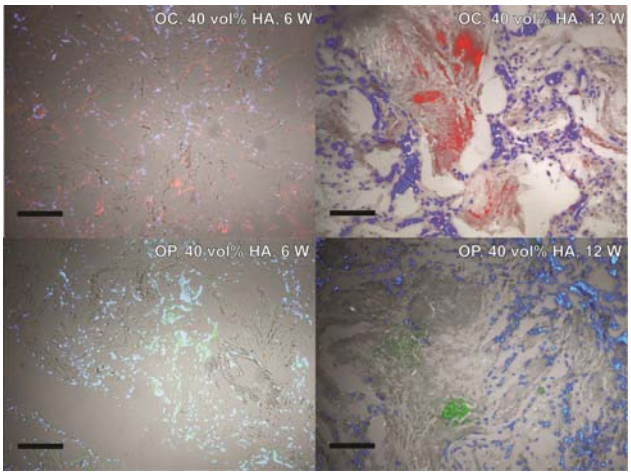

Figure 2. Col-HA scaffold explants stained positively for OC (red) and OP (green) after 6 and 12 weeks. Scale bar = 100 μm.

Conclusions: Novel Col-HA scaffolds with improved architecture and mechanical properties supported osteogenesis in a subcutaneous ectopic site where the recruitment of osteoprogenitor cells is impaired. Significantly, osteogenesis was observed even in the absence of exogenous osteogenic growth factors, suggesting the potential of these biologically-derived scaffolds as an improved synthetic bone graft substitute or tissue engineering scaffold.

References:

1. Calori GM. Injury. 2011;42Suppl 2:S56-S53.
2. Kane RJ. Biomaterials. 2014, (submitted).
3. Weiss HE. Tissue Eng Part A. 2012;18:1334-43.