Biologically-Inspired Hydroxyapatite-Collagen Scaffolds Support Osteogenesis in an Ectopic Murine Model

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Statement of Purpose: Current synthetic bone graft substitutes provide suitable osteoconductivity but are not sufficiently osteogenic, osteoinductive, or mechanically robust for surgical handling, fixation, and load-bearing [1]. We have developed biologically-inspired collagen-hydroxyapatite (Col-HA) scaffolds with order of magnitude improvements in permeability and mechanical properties compared to conventional lyophilized scaffolds, providing a microenvironment designed to support osteogenesis [2]. Therefore, the objective of this study was to evaluate the osteogenicity of these scaffolds in an 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, as described previously [2]. Scaffolds in this study were prepared with a porosity of 85 vol% and either 40 vol% HA in phosphate-buffered saline (PBS), 40 vol% HA in fetal bovine serum (FBS), 20 vol% HA in PBS, or 0 vol% HA in PBS (n = 8/group). Scaffolds were implanted ectopically in eight, 4 week old female athymic nude mice [3]. Four mice were sacrificed at 6 and 8 weeks. Following fixation, explanted scaffolds were imaged via micro-CT (µCT-80, Scanco) to quantify the volume of new bone formation. Explants were subsequently, embedded, sectioned, and stained with H&E and Masson's trichrome for histology.

Results: The bone volume measured by micro-CT increased with increasing HA content, and from pre-implantation to post-implantation in all Col-HA scaffolds at 6 and 12 weeks, but was not significantly increased in collagen scaffolds (Fig. 1). The processing media had no effect on the bone volume. Note that the non-statistically significant decrease in bone volume between 6 and 12 weeks for HA reinforced collagen scaffolds was not present when normalized to the total scaffold volume.

All scaffold explants exhibited complete cellular infiltration and significant matrix deposition after 6 weeks implantation (Fig. 2). At 6 weeks, scaffolds prepared with 40 vol% HA, no matter whether processed in PBS or FBS, exhibited dense cell populations and matrix deposition, as well as vascularization (Fig. 2). Scaffolds with less than 40 vol% HA exhibited no evidence of angiogenesis. Collagen scaffolds exhibited smaller cell populations and less dense matrix deposition compared to Col-HA scaffolds. By 12 weeks, the porous architecture of scaffolds with 40 vol% HA was rearranged by infiltrating cells. In contrast, the original scaffold architecture was mostly maintained at 12 weeks in collagen scaffolds. By 12 weeks, regions of the scaffold that were vascularized maintained healthy cell populations, while regions without vasculature exhibited a decrease in cell number in both collagen and Col-HA scaffolds (Fig. 2).

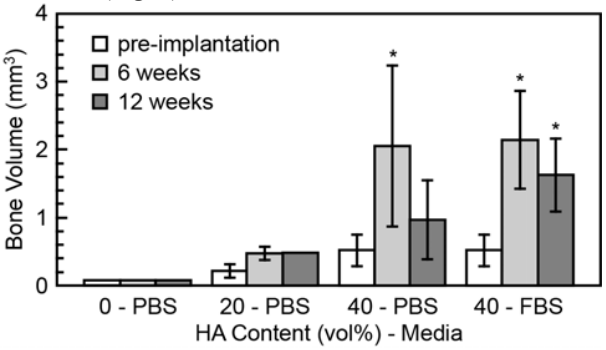

Figure 1. Bone volume in ectopically implanted scaffolds. Col-HA scaffolds showed an increase in bone volume after 6 weeks, which increased with increasing HA content. * $p < 0.05$ vs. pre-implantation, Wilcoxon.

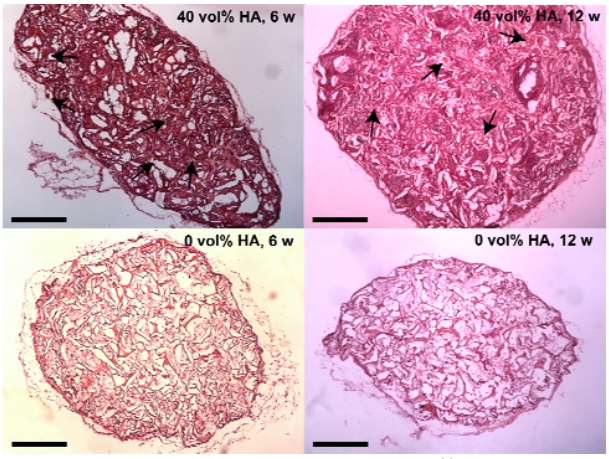

Figure 2. Collagen and HA-Col scaffolds were completely infiltrated by cells after 6 weeks post-implantation but only scaffolds with 40 vol% HA were vascularized (arrows). Scale bar = 500 µm.

Conclusions: Novel Col-HA scaffolds possessing improved mechanical properties and architecture were able to form bone in an ectopic site where the recruitment of osteoprogenitor cells is impaired. Moreover, the presence of HA promoted angiogenesis prior to osteogenesis. Significantly, angiogenesis and osteogenesis were observed even in the absence of exogenous angiogenic and osteogenic growth factors, suggesting the potential of these biologically-inspired 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. 9th World Biomaterials Congress. 2012.
3. Weiss HE. Tissue Eng Part A. 2012;18:1334-43.