Hydroxyapatite Coating of Porous Polycaprolactone to Enhance Integration of a Tissue-Engineered Total Disc Replacement

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Introduction

Endplates form the interfaces between the intervertebral discs of the spine and the adjacent vertebral bodies. These structures consist of a thin layer of hyaline cartilage and an adjacent layer of cortical bone.[1] With aging or following injury, degeneration of the intervertebral discs and adjacent endplates is common and is frequently associated with back pain.[2] There is a significant need to develop new treatment strategies to address both the disc and vertebral endplate. Towards this end, our group developed tissue engineered total disc replacements with endplates (endplate modified disc like angle-ply structures, eDAPS) for the treatment of severe, advanced-stage disc and endplate degeneration. In contrast to other designs for tissue engineered whole discs, the porous polymer endplate analog of the eDAPS provides an interface through which integration of the engineered disc with the native vertebral body can occur.[3] However, our previous animal studies demonstrated that robust mineralization of this interface was not present after 20 weeks *in vivo*. The purpose of this study was to optimize the design of the endplate region, via the inclusion of a hydroxyapatite (HA) coating, to improve endplate mineralization and eDAPS integration following *in vivo* implantation.

Methods

Scaffold Fabrication and HA coating

Porous poly(ε-caprolactone) (PCL) scaffolds were fabricated via a salt leaching method, as previously described, to generate constructs 4mm in diameter and 1.5 mm thick.[3] To coat the PCL scaffolds in HA, foams were hydrated through a gradient of ethanol, followed by serial overnight immersions in 2M NaOH and simulated body fluid (SBF).[4]

In Vitro Studies

Prior to cell seeding, PCL only scaffolds and HA coated PCL scaffolds were hydrated and sterilized through an ethanol gradient and coated overnight in fibronectin. P2 bovine bone-marrow derived mesenchymal stem cells (MSCs) were seeded on the top and bottom surface of each scaffold at a density of 3,333 cells/mm^2. MSC-seeded scaffolds were cultured in either basal or osteogenic media (n=4 per group) for 5 weeks. At the end of the culture duration, construct viability (MTT assay) and alkaline phosphatase activity (ALP, Sigma Aldrich kit) were quantified. Additional samples (n=3 per group) were cryosectioned in the sagittal plane and stained for calcium deposits using a Von Kossa staining kit (Abcam).

In Vivo Studies

For *in vivo* evaluation of the HA coating, PCL scaffolds 4mm in diameter and 5mm thick were fabricated, to mimic the size of the eDAPS previously evaluated our rat tail disc replacement model. In accordance with our approved IACUC protocol at the Corporal Michael J. Crescenz VA Medical Center, the tail disc spaces of five athymic rats were implanted with acellular PCL (n=2) or HA-coated PCL scaffolds (n=3), using our previously described surgical procedure and external fixator.[3] Briefly, the native C8-C9 tail disc space was removed, and a partial corpectomy of the adjacent vertebral bodies was performed with a high-speed burr, such that the constructs could be placed in apposition with the marrow of the vertebral bodies. After 10 weeks, animals were euthanized and vertebral body-scaffold-vertebral body motion segments harvested for analysis. Motion segments were fixed in formalin and subjected to μCT scanning at 10μm resolution to visualize the three-dimensional tissue distribution within the scaffold following *in vivo* implantation. Samples were then decalcified and processed for paraffin histology. Histologic sections were stained with the Mallory-Heidenhain trichrome stain to distinguish unmineralized collagen (blue) from mineralized collagen (pink), and immunohistochemistry (IHC) was performed for osteocalcin. Significant differences ($p<0.05$) between groups were assessed via an ANOVA with Tukey's post-hoc test.

Results

In vitro studies of HA coated and PCL only scaffolds seeded with bone marrow-derived MSCs demonstrated a significant increase in

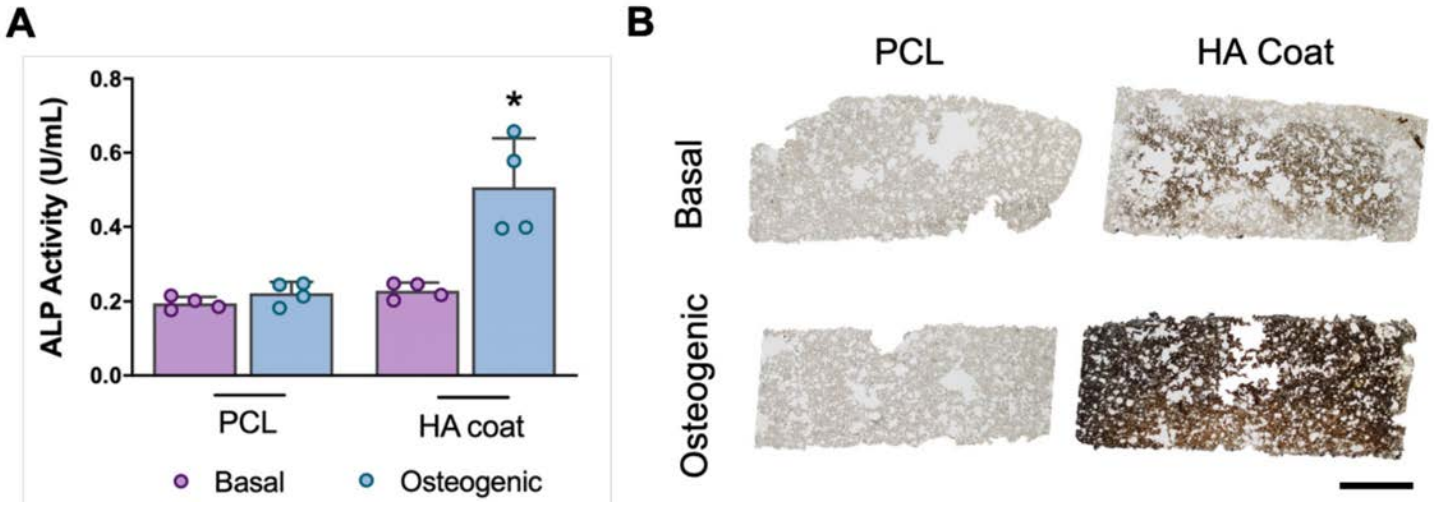

Figure 1. (A) ALP activity and **(B)** Von Kossa staining of HA coated or PCL only foams seeded with MSCs and cultured for 5 weeks. * 5 p,0.05 compared to all other groups. Scale 5 1 mm.

construct ALP activity in the HA-coated group cultured in osteogenic media (Figure 1A). There were no statistically significant differences in MTT absorbance across groups. Von Kossa staining of the constructs suggested increased calcium deposition in the HA coated group compared to the PCL only group, in both basal and osteogenic media culture conditions (Figure 1B). *In vivo*, collagenous matrix deposition occurred within the initially acellular scaffolds in both groups after 10 weeks implantation. There was increased immunohistochemical staining for osteocalcin within HA coated scaffolds compared to the PCL only controls (Figure 2, left panel). Additionally, the Mallory-Heidenhain trichrome stain revealed areas of mineralized collagen (pink staining) present in the HA coated group that were not present in the uncoated group (Figure 2, middle panels). 3D μCT reconstructions of the constructs 10 weeks post-implantation demonstrated increased mineralized tissue deposition with HA coating of PCL scaffolds (Figure 2, right panel).

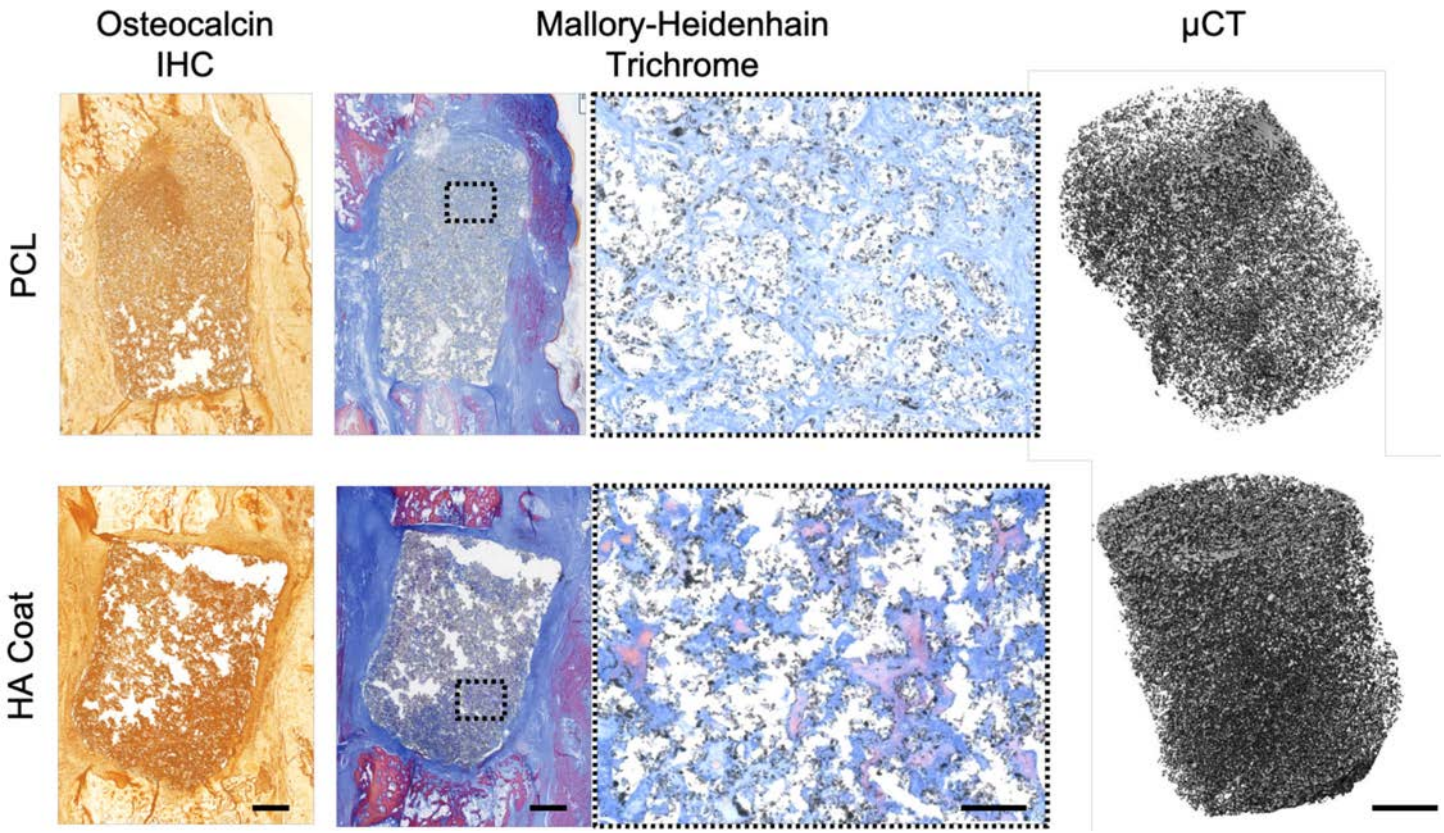

Figure 2. IHC for osteocalcin (scale 5 1mm), staining with the Mallory-Heidenhain trichrome stain (left scale 5 1mm, right scale 5 100μm), and μCT (scale 5 1mm) of acellular PCL and HA coated foams implanted in the rat caudal spine for 10 weeks.

Discussion

The results from our *in vitro* and *in vivo* experiments suggest that coating of porous PCL scaffolds with HA can increase their osteoinductive potential. In our previous work, where eDAPS with PCL only endplates were implanted in the rat caudal disc space, mineralized collagen was not observed within the endplate region until 20 weeks post-implantation.[3] Here, we observed staining for mineralized collagen within the construct at 10 weeks post-implantation in the HA coated group, suggesting that integration may be accelerated by the HA coating. Our current findings are consistent with previous work in the fracture repair field, where hydroxyapatite coating by other methods improved *in vitro* and *in vivo* osteogenesis.[5,6] Ongoing work is investigating the mechanical strength of the integration of the HA coated PCL with the native vertebral body, and the inclusion of macroscopic channels within the constructs to further promote integration. In the future, these HA coated PCL foams will be utilized for the endplate region of the engineered disc, and integration and bone formation assessed in small and large animal models.

Significance/Clinical Relevance

This design modification to our tissue engineered endplate and intervertebral disc replacement has the potential to improve and accelerate integration of the construct with the native vertebral bone, which will be critical for clinical translation.

Acknowledgments

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