Developing a Biologic Replacement for the Porcine Accessory Carpal as a Model for the Treatment of Thumb OA

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Introduction

Trapeziometacarpal (TMC) osteoarthritis (OA) is one of the most common conditions affecting middle and older aged adults[1]. Given that the thumb is central to all activities of daily living, loss of function has a significant impact on quality of life. Patients with TMC OA are initially managed with activity modification, NSAIDs, splinting, and corticosteroid injections[2]. These treatments fail in the long term, however, and many patients eventually require destructive surgical intervention, involving removal of all or part of the trapezium, and replacement with tendon, fascia, or an artificial implant[2]. While effective at reducing pain, these procedures compromise grip strength and, in some cases, result in subsidence and disFigurement of the hand[2]. Efforts to replace articular cartilage (and bone) with living, functional tissue have matured substantially over the last two decades[3], as has technology for generating constructs that can match the anatomical complexity and geometry of native articulating surfaces[3,4]. For these technologies to progress towards translation, appropriate large animal models are required. In our previous work, we identified the porcine accessory carpal (AC) as a potential model for TMC OA, given its similar shape, size, and loading[5]. Here, we develop strategies for the fabrication of tissue engineered biologic replacements for the porcine AC bone and cartilage, and demonstrate feasibility.

Methods

Eight AC bones were isolated from the right forelimbs of adult Yucatan minipigs and four human trapezia were isolated from cadaveric donors. Samples were fixed in formalin and imaged via μCT (VivaCT 75, Scanco), before and after immersion in Lugol's solution (5% I_2, 10% KI in water) to enhance cartilage contrast. DICOMs from the initial scan were imported into ITK-SNAP[6] and bone segmented. A surface mesh was exported to Meshlab (ISTI) for simplification and Solidworks (Dassault Systèmes) to render 3D objects and compute bone volume and surface features. Post-Lugol's scans were manually registered with the bone and processed similarly, with the cartilage layer segmented in a semi-automated manner. After imaging, samples were decalcified, processed into paraffin, sectioned, and stained with Safranin-O/fast green to visualize cartilage, bone, and fibrous tissue and Picrosirius red, to visualize collagen. Immunohistochemistry was used to assess distribution of collagen II. In Solidworks, an implant of the articulating cartilage surface and first third of the AC bone was designed. A cylindrical peg was included for fixation into the parent bone. Negative molds were designed for both the bone portion and the composite implant. The bone mold was 3D printed out of aluminum alloy using direct metal laser sintering (DMLS), and the composite mold was 3D printed in an ABS-like photopolymer. To form the bone integrating portion of the construct, poly(ε-caprolactone) (PCL) was dissolved in chloroform at 20% wt/vol and mixed with NaCl crystals sieved to ~106 μm with inclusion of Zirconium nanoparticles for radioopacity. The slurry was poured into the bone mold and the solvent was evaporated for 5 days. The units were demolded and washed in distilled water to remove salt. The resultant construct was imaged via μCT (μCT50, Scanco medical). As a proof of concept, a 5 wt% agarose solution doped with red food coloring for visualization was poured into the composite mold, and the PCL bone integrating component was added to shape the cartilage portion. Finally, a 1% wt/vol methacrylated hyaluronic acid (MeHA) solution with 20 million juvenile bovine mesenchymal stem cells (MSCs) per mL was dispensed into the composite mold and the porous bone component placed into the mold to form the final shape of the cartilage. Gelation occurred via inclusion of APS/TEMED, with entire mold placed at 37°C for 10 minutes. After 24 hours in culture, construct viability was assessed.

Results

The porcine AC bone shows marked anatomical similarity to the human trapezium in both its size and saddle shape of its major articulating surface. (Figure 1A, 1B) Both species show strong staining for proteoglycans on their cartilage surfaces (Figure 1C), and collagen throughout the tissue depth (Figure 1D). Type II collagen is high in the cartilage surface of both the human trapezium and porcine AC (Figure 1E). Using surface meshes generated from μCT, an implant was designed to replace the full

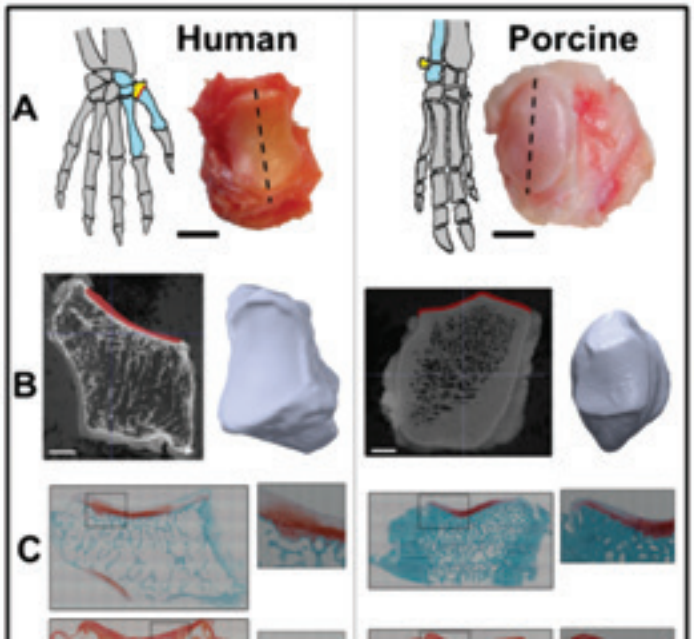

Figure 1. **(A)** Diagram and gross view of the human trapezium and porcine AC. Dotted line represents sectioning plane. Scale = 3mm. **(B)** μCT slices in ITK-SNAP showing cartilage segmentation in red and 3D renderings in Solidworks. Scale = 3mm. **(C)** Safranin-O/fast green. **(D)** Picrosirius Red. **(E)**. Immunohistochemistry for collagen II. Scale = 3mm (1mm for insets).

articulating surface of the porcine AC. (Figure 2A) The boney portion of this implant was generated using PCL foam (Figure 2B-D). MicroCT showed that this recapitulated the geometry of the original design yielding a volume that was 76% similar to the template (Figure 2E). A second mold with both the bone-mimicking and cartilage portion of the implant formed a combined implant (Figure 2F). When cast into this composite, MSCs remained viable (Figure 2G).

Discussion

In this study, we evaluated the histological and geometric properties of the porcine AC compared to the human trapezium. The two showed remarkable anatomic and compositional similarities. Furthermore we developed a method to fabricate a composite implant, mimicking the boney (using PCL foam) and cartilage (using a hydrogel) regions of the AC. Future work will improve integration between the hydrogel and foam and

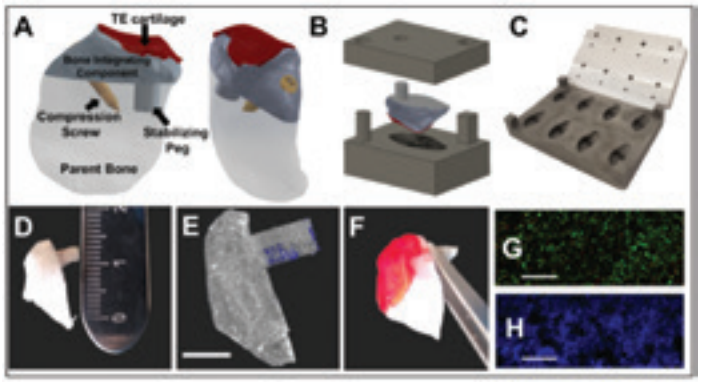

Figure 2. (A) Design of composite implant for biologic resurfacing of the porcine AC**. (B, C)** Mold for fabrication of implant. **(D)** PCL foam AC **(E)** μCT scan of construct. Scale = 5mm **(F)** Composite construct with PCL foam and hydrogel (red) **(G)** Live (green)/Dead (red) image of MSCs in MeHA in the cartilage layer. Scale = 200μm. **(H)** Confocal image of PCL foam (blue is auto-fluorescence of PCL). Scale = 200μm.

refine methods for maturing the construct, to establish means for functional biologic joint resurfacing in a large animal model.

Significance

This study compared the porcine accessory carpal and human trapezium and refined strategies for the fabrication of tissue engineered osteochondral implants for biologic joint resurfacing, providing a route for effective biologic joint replacement for patients with TMC OA.

Acknowledgments

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References

1. Becker+, *CORR*, 2013.
2. Wajon+, *Cochrane Database*, 2015.
3. O'Connell+ *J Knee Surg*, 2012.
4. Saxena+ *Tissue Eng*, 2016.
5. Stoeckl+, *ORS* 2018.
6. Yushkevich+, *Neuroimage*, 2006.