Aseptic Tissue Machining of Fresh (Viable) Canine Patellar Osteochondral Allografts for Bending

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INTRODUCTION: Osteochondral allografts (OCAs) have been used with increasing regularity in recent years to treat defects in articular cartilage, but the difficulty of matching donor and recipient articular surface curvature provides significant challenges when sourcing donor allografts from tissue banks. To address this concern, our lab has introduced bendable osteochondral allografts (BOCAs), which enable modification of allograft articular surface curvature by machining grooves through the full depth of the subchondral bone.[1] To address the feasibility of implementation of this technique in a realistic clinical setting, we used a canine preclinical model to investigate the functionality of BOCAs, and whether fresh (viable) OCAs could be safely recovered, shipped, processed, machined, and transplanted without compromising tissue asepsis, viability, or survivability.

METHODS: With IACUC approval (#16680), ten patellae were recovered from the stifle (knee) joints of five adult purpose-bred canine tissue donors at the Thompson Laboratory for Regenerative Orthopedics (University of Missouri, Columbia, MO). Samples were stored and shipped overnight in Missouri Osteochondral Allograft Preservation System (MOPS®) media to the Musculoskeletal Biomechanics Lab (Columbia University, New York, NY). Whole canine patellae were transferred in a cell culture hood under aseptic conditions into custom-built rigid metal-free autoclavable polysulfone fixation rigs (Figure 1). The samples were secured into sterilized single-use PLA inserts with Adafruit low-temperature fixation thermoplastic so the tissue could be held still during machining without compromising tissue integrity or access to media. The fixation rigs were sealed for transit outside sterile environments with an internal neoprene rubber gasket and an external layer of Parafilm. Fixed patellae were scanned at an isotropic 78 μm voxel resolution using a VivaCT 80 microCT scanner in the Bone Bioengineering Lab (Columbia University, New York, NY). Patella scans were processed using the open-source imaging software 3D Slicer to create volumetric models of the patellae within the fixation rigs, and sample-specific CNC milling machine cutting paths were generated in the SOLIDWORKS HSMworks application (autodesk.com). The time between receiving the patellar samples and completing the machining subject-specific file paths took five days in total: one day for mounting, two days for microCT scanning samples and generating DICOM files, and two days for processing the DICOM files and preparing the machining toolpaths. Machining was performed in a purpose-built laminar airflow machining cabinet with UV sterilization cycles run between samples. A custom acrylic machining fixture was used to mount the fixation rigs on the CNC, creating a shared machining window for all samples. For the machining, cutting settings were chosen to minimize heat generated through the milling process, and the MOPs® reservoir acted as a cutting fluid throughout the machining process. Shell allograft samples took 25 minutes on average to plane and BOCA samples included an additional 30-minute grooving process. All patellae were planed on their anterior side to a total depth of 6 mm, creating shell osteochondral allografts, and the left patellae went through an additional machining step, where 2 mm wide grooves were milled through the subchondral bone to the basal surface of the articular cartilage to create BOCAs. Samples were removed from their holders and shipped back to Missouri, the surgical center for aseptic transplantation. Fragments of the allograft samples and MOPS® media aliquots were sent to the Veterinary Medical Diagnostic Laboratory (University of Missouri, Columbia, MO) for aerobic and anaerobic culture before implantation to check for clinically relevant microbial growth. The total time between retrieval of patellae from the dogs and transplantation as allografts was 33 days.

RESULTS: Canine patellae underwent shipment and processing to create standard shell and BOCA allografts while maintaining asepsis and viability. No gross damage was noted in the articular surfaces of the allografts following machining. The results of direct aerobic and anaerobic microbial cultures showed no microbial growth in the remnant patellar allograft tissue or MOPS® media samples of the allografts at any time point.

DISCUSSION: Fresh (viable) osteochondral allografts were successfully recovered, stored, shipped across states, scanned, machined, shipped back, and then transplanted into live donors without compromising allograft asepsis or viability. Results reported last year showed that the machining process and bending did not compromise chondrocyte viability in similar patellar allografts.[2] The microbial culture results of transported and processed allograft samples add greater confidence to those prior viability results, demonstrating clinical feasibility for aseptic grooving of osteochondral samples to enable tissue banks to perform this type of CNC machining of donor osteochondral tissue followed by safe transport of the machined allografts to hospitals nationwide.

SIGNIFICANCE/CLINICAL RELEVANCE: The success of the method implemented for CNC machining of fresh (viable) osteochondral allografts allows for grooving and standard transport of recovered allografts to enable the transplantation of bendable OCAs without compromising tissue asepsis and viability.

REFERENCES: 1. Peterson CA et al. *Journal of Biomechanics* (2022) 2. Spack KA et al. *Orthopedic Research Society Annual Conference* (2023)

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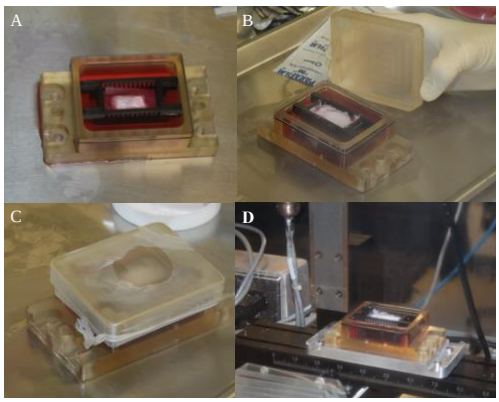

Figure 1: Fixation of allografts for machining **A**: A canine patella is placed in the PLA insert within the machining rig in a bath of MOPS® media. **B**: A patella is secured within fixation rig using low-temperature thermoplastic. **C**: The machining rig is sealed with parafilm for transportation out of the sterile enclosure. **D:** The machining fixture holds the machining rig during CNC milling.

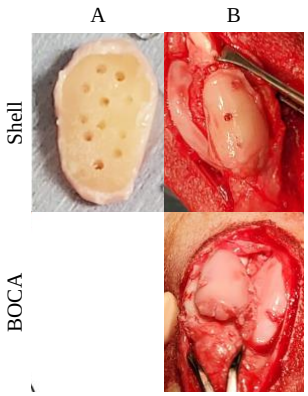

Figure 2: Shell and bendable allografts during surgical insertion. **A:** The deep bony surface of allografts. **B:** The articular surface shows intact cartilage during insertion.