**High-Fidelity Tissue Engineering of Patient Specific Auricles for Reconstruction of Pediatric Microtia**

Alyssa J. Reiffel, MD; Bryan N. Brown, PhD; Karina A. Hernandez, DO; Sherry Zhou, MEng; Lawrence J. Bonassar, PhD; Jason A. Spector, MD

**Abstract**

**Background:** We sought to combine digital photogrammetry with computer-assisted design/computer-assisted manufacturing (CAD/CAM) techniques to develop biocompatible tissue-engineered auricular reconstructions that would more closely mimic the normal anatomy of the patient-specific external ear and the complex mechanical behavior of native elastic cartilage, while avoiding the morbidity and poor aesthetic outcomes typically associated with traditional reconstructions.[1-5]

**Methods:** The three-dimensional structures of normal pediatric ears were digitized using the Cyberware® 3D Digitizer. Images were converted to virtual solids using Geomagic Studio® and translated into volume models for mold design. Image-based synthetic reconstructions of these ears were fabricated under sterile conditions from collagen type I hydrogels cast from these three-dimensional molds. One group of constructs was seeded with 2.5x10^7 bovine auricular chondrocytes. Cellular and acellular constructs were implanted subcutaneously in nude rats and harvested after 4weeks.

**Results**: Post-implantation, cellular constructs effectively maintained the anatomical features of the external ear including tragus, lobule, helix, and antihelix. Post-harvest weight of cellular specimens was significantly greater than that of acellular specimens (4.2±0.29g v. 0.80±0.12g, p=<0.05). Safranin O-staining revealed that only cellular constructs demonstrated evidence of a self-assembled perichondrial layer and cartilage deposition by lacunar chondrocytes (Figures 1-2). Verhoeff staining of cellular constructs revealed elastin fibers interspersed among the chondrocytes. The confined compression modulus of cellular constructs increased significantly from 9.2±1.4kPa pre-implantation to 31±14kPa at 4weeks (p<0.05).

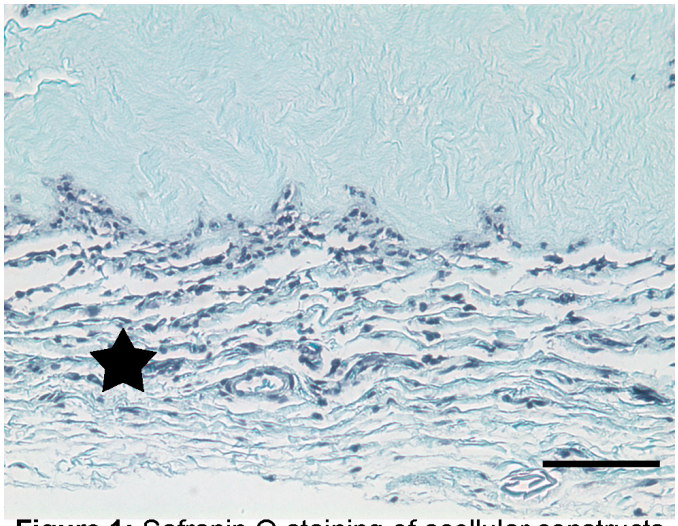

**Figure 1:** Safranin O-staining of acellular constructs demonstrated a thin peripheral capsule containing fibroblast-appearing cells (*star*), but no evidence of collagen deposition. *Scale bar = 100μm.*

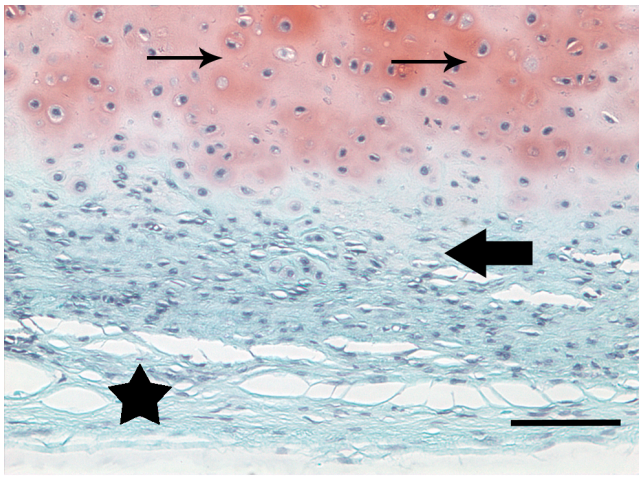

**Figure 2:** Safranin O-staining of cellular constructs similarly demonstrated a thin peripheral capsule containing fibroblast-appearing cells (*star*). However, only cellular constructs demonstrated evidence of a self-assembled perichondrial layer (*large arrow*) or cartilage deposition by chondrocytes in their lacunae (*small arrows*). *Scale bar = 100μm.*

**Conclusions:** We have successfully combined digital photogrammetry with CAD/CAM techniques to create biocompatible *patient-specific* human-sized tissue-engineered constructs for ear reconstruction. The cellular constructs' life-like biomechanical properties and maintenance of volume, shape and topographical characteristics over time can be attributed in part to their unique type I collagen composition. Furthermore, this material chemistry allows for chondrocyte survival and the *in vivo* deposition of elastic cartilage. We believe our approach to auricular tissue engineering holds tremendous promise for translation to the clinical realm.

**References**


1. Shieh, S.J., Terada, S., Vacanti, J.P. Tissue engineering auricular reconstruction: in vitro and in vivo studies. Biomaterials 25: 1545-1557, 2004.
2. Bichara, D.A., O'Sullivan, N.A., Pomerantseva, I., et al. The tissue-engineered auricle: past, present, and future. Tissue Eng Part B Rev 18: 51-61, 2012.
3. Haisch, A., Klaring, S., Groger, A., et al. A tissue-engineering model for the manufacture of auricular-shaped cartilage implants. Eur Arch Otorhinolaryngol 259: 316-321, 2002.
4. Rodriguez, A., Cao, Y.L., Ibarra, C., et al. Characteristics of cartilage engineered from human pediatric auricular cartilage. Plast Reconstr Surg 103: 1111-1119, 1999.
5. Liu, Y., Zhang, L., Zhou, G., et al. In vitro engineering of human ear-shaped cartilage assisted with CAD/CAM technology. Biomaterials 31: 2176-2183, 2010.


**Disclosure/Financial Support**


This work was supported in part by the Ruth L. Kirschstein National Research Service Award (T32 HL083824-05) Institutional Research Training Grant (Dr. Reiffel).

None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript.