Utilizing a Novel Cell Sourcing Strategy to Fabricate the First Full-Scale Tissue Engineered Human Ear Scaffold

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Disclosure/Financial Support: Dr. Lawrence J. Bonassar is co-founder and equity holder of 3D-BioCorp.

INTRODUCTION: Previously, we fabricated patient-specific auricles using bovine auricular chondrocytes, which displayed effective permanence with structural, biochemical, and mechanical properties similar to native auricular cartilage after 6 months *in vivo.* However, autologous tissue donation generates a limited cell yield, and further expansion of donor cells can negatively impact chondrogenic capacity. To overcome this challenge, we sought to generate human auricular cartilage through the combined implantation of human auricular chondrocytes (hAuC) with human mesenchymal stem cells (hMSC) as a novel cell sourcing strategy in order to fabricate the first full-scale human ear.

MATERIALS AND METHODS: First, hAuC from discarded otoplasty specimens (1mg of donor ear cartilage) and bone-marrow derived hMSC were encapsulated into type I collagen hydrogels in ratios of 100:0, 50:50, and 0:100 hAuC:hMSC with a cell density of 25 million cells/mL. 8mm diameter constructs were implanted subcutaneously in the dorsa of nude mice, and harvested at 1 and 3 months for analysis. Subsequently, the 50:50 hAuC:hMSC mixture was used to fabricate full-scale ear constructs using the same cell sources. Utilizing digital photogrammetry and computer-assisted design/computer-aided manufacturing technology, three-dimensional structures of a normal pediatric ear were digitized and converted to a virtual solid for mold design. Image-based synthetic reconstructions of these ears were produced from collagen type I hydrogels seeded with hAuC and hMSC. Full scale-ear constructs were then implanted subcutaneously into the dorsa of nude rats, and harvested after 3 months.

RESULTS: 50:50 hAuC:hMSC 8mm scaffolds developed an auricular cartilage microstructure, including organized perichondrium composed of collagen, a rich proteoglycan matrix, cellular lacunae, and a dense elastin fiber network. Biochemical analysis confirmed that mixed cell 8mm constructs featured significantly more proteoglycan content than the 100% hMSC group; proteoglycan content increased significantly between 1 and 3 months. Finally, upon 3-month explant, gross analysis of full-scale human ear constructs showed maintained shape, projection, and flexibility with a small degree of contraction.

CONCLUSION: Co-implantation of hAuC with hMSC in a 50:50 ratio produces human auricular cartilage that is indistinguishable from native auricular cartilage, and can be used as a viable strategy to produce a full-scale pediatric human ear construct. Using only 1mg of donor ear cartilage, we have successfully fabricated the first human full-scale, patient-specific tissue engineered ear scaffold, which holds exceptional promise for clinical translation.