Co-Implantation of Auricular Chondrocytes and Mesenchymal Stem Cells for Auricular Cartilage Generation

Benjamin P. Cohen, Ope A. Asanbe, Jason A. Spector, Lawrence J. Bonassar

Section: Dental and Craniofacial

The encapsulation of bovine auricular chondrocytes (AuCs) in collagen has successfully formed auricular cartilage *in vivo*, however this technique requires a large number (>200x10^6) of cells. Human AuCs can be isolated from donor tissue but lose chondrogenic potential when cultured beyond initial passages. Mesenchymal stem cells (MSCs) can differentiate into chondrocytes and have been shown to promote cartilage formation when cultured with articular chondrocytes. Here, human AuCs and MSCs were mixed with 10mg/mL type I collagen hydrogels in ratios of 100:0, 50:50, and 0:100 AuC:MSC at a density of 25x10^6 cells/mL. Disc constructs 2mm thick by 8mm diameter were implanted subcutaneously in nude mice for 1 and 3 months. Gross inspection showed that 100% AuC and 50:50 constructs developed cartilage-like color and appearance at 1 and 3 months, while 100% MSCs contracted significantly after 3 months. Safranin-O and Verhoeff's staining to analyze GAG content and elastin fibers, respectively, revealed the deposition of both molecules in 50:50 constructs at 1 month and increased presence at 3 months in 100% AuC and 50:50 constructs. Picrosirius red staining of 50:50 mixed cell constructs displayed a collagen-rich perichondrial layer characteristic of auricular cartilage by 3 months. The maintenance of size, the deposition of elastic cartilage molecular components, and the organization of auricular cartilage microstructure demonstrates the success of co-implantation of MSCs with AuCs for the formation of auricular cartilage using 50% fewer chondrocytes. The use of MSCs to supplement chondrocytes is a critical step toward the clinical viability of tissue engineered auricular reconstruction techniques.