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Lecture

A pilot study comparing mechanical properties of tissue-engineered cartilages and various endogenous cartilages

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ABSTRACT

Background: Mechanical properties of tissue-engineered cartilage and a variety of endogenous cartilage were measured. The main goal was to evaluate if the tissue-engineered cartilage have similar mechanical characteristics to be replaced with rib cartilage in microtia reconstruction. Such study lays the foundation for future human clinical trials for microtia reconstruction.

Method: Atomic force microscopy and compression testing were used to measure the viscoelasticity of tissue-engineered cartilage (stem cell seeded on Poly lactic co-glycolytic acid nanofibers and Pellet) and endogenous cartilage: conchal bowl, microtic ears, preauricular remnants, and rib. Atomic force microscopy, calculates biomaterial elasticity through force-deformation measurement and Hertz model. Compression testing determines the stress relaxation by measuring slope of stress reduction at 10% strain.

Finding: Tissue-engineered cartilage demonstrated elasticity (4.6 kPa for pellet and 6.6 kPa for PLGA) and stress relaxation properties (7.6 (SD 1.1) kPa/s for pellet) most similar to those of native conchal bowl cartilage (31.8 (SD 18) kPa for the elasticity and 15.1 (SD 2.1) kPa/s for stress relaxation factor). Rib cartilage was most dissimilar from the mechanical characteristics of conchal cartilage and demonstrated the highest elastic modulus (361 (SD 372) kPa). Moreover, except preauricular cartilage samples, the level of elastic modulus increased with age.

Interpretation: The use of tissue-engineered cartilage developed via PLGA and Pellet methods, may be an appropriate substitute for rib cartilage in the reconstruction of microtic ears, however their mechanical characteristics still need to be improved and require further validation in animal studies.

1. Introduction

The current treatment for patients with microtia (deformed external ear) and anotia (absent external ear) involves harvest and sculpting of rib cartilage with implantation under the skin. Over time the reconstructed ear is subjected to staged procedures for final ear shaping (Baluch et al., 2014; Quatela et al., 2006). Fig. 1 shows the reconstruction steps on a patient. The final result resembles an external ear, however, the rib cartilage (a hyaline cartilage) construct lacks the flexibility of native ears (Brent, 2002). A solution to this limitation may be found in tissue engineered (TE) cartilage using mesenchymal stem cells (MSCs) from a source such as the umbilical cord (UC). As shown by our previous study, UC MSCs can be chondroinduced to demonstrate an elastic cartilage phenotype, with deposition of extracellular glycosaminoglycans (GAGS), appropriate ratios of collagen II to I mRNA (> 1), with increased elastin mRNA and protein. The staining patterns for collagen I, II, X, and elastin are also comparable to normal conchal bowl elastic cartilage (Dahl et al., 2011; Pappa et al., 2014). However still a major concern with any implanted engineered elastic cartilage is the capacity to maintain an elastic phenotype, and the capacity to resist the deforming forces of the healing wound, primarily skin contracture. To date, all implanted TE cartilage has the tendency to become hypertrophic and eventually to ossify (Kachanov, 2004; Mow et al., 1980). For any TE cartilage to successfully replace rib cartilage as the primary source of cartilage for microtia reconstruction, this inexorable process toward ossification must be controlled. The second issue is the capacity to resist the deformation of the healing wound: an overly elastic cartilage will condense into a compact mass without recognizable detail; a non-elastic cartilage becomes no better than the inelastic rib cartilage it is trying to replace. In the final analysis, a combination of these qualities may be required; greater inelasticity early during the healing process, and greater elasticity when healing is complete (Balaji, 2015;

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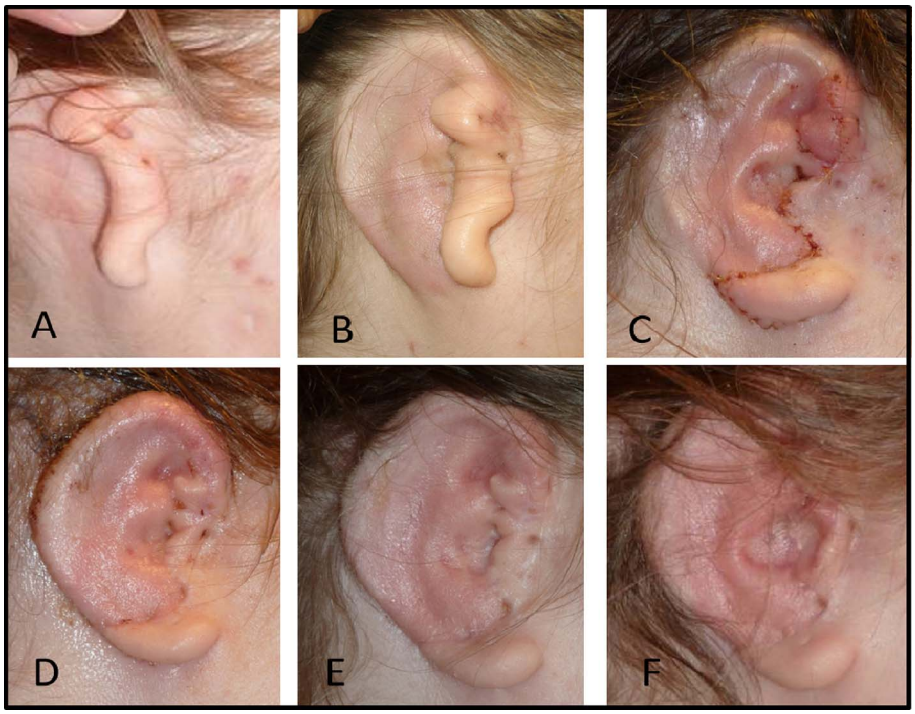

Fig. 1. A panel of pictures depicting a patient with a right lobular microtia and the staged surgical treatment: A) prior to surgery, B) after the first stage of cartilage harvest and implantation, C) lobule transfer, D) release of the framework with skin grafting to the posterior construct, E) four months later, and F) after conchal bowl creation.

Blanco, 2012).

Cartilage in particular exhibits strain-rate dependent properties because of the complexity of its microstructure. To a first approximation, cartilage can be considered an elastic matrix, similar to a sponge that contains a fluid. When deformed, the cartilage responds at the microscopic level both by elastic deformation of the porous extracellular matrix as well as by flow of the fluid through the matrix. The fluid within the matrix resists being forced to flow through the matrix which is dependent on the rate of applied strain, in this way behaving like a *viscoelastic* material (Meyers and Chawla, 1999; Perzyna, 1966).

The goal of the study is to define the viscoelastic properties of endogenous cartilage sources, and to compare them to TE cartilage generated from UC MSCs. Such a study provides information whether the TE cartilage will resemble the native cartilage of the ear to determine whether the TE cartilage is a good candidate for ear reconstruction. Although some literatures discuss the privilege of TE cartilage on microtia reconstruction, they don't provide any information regarding the mechanical characteristics of the endogenous and TE cartilage used on microtia reconstruction. Such information is extremely important for the perception of the microtia reconstruction process. The longer-term goal is to lay the foundation for large animal studies and clinical trials to test our TE cartilage in vivo.

2. Methods

In order to determine the biomechanical properties of UC MSC-derived elastic cartilage, we utilized Atomic Force Microscopy (AFM) and compression testing to determine elasticity and stress relaxation rates of the following cartilage samples: TE on 2D surfaces and 3D nanofibers, conchal bowl, rib, preauricular remnants (which occur in 1% of the population), and abnormal microtic samples. The elasticity and stress relaxation rate parameters delineate the viscoelastic characteristic of the material (Kachanov, 2004; Mow et al., 1980; Protsenko et al., 2008). The independent variable in this study is “cartilage type” while dependent variable is “elastic modulus” and “stress relaxation rate”.

Human samples were obtained from patients undergoing facial reconstructive surgeries. Discarded human cartilage from conchal bowl, rib, preauricular remnants, and microtia were collected with IRB approval (IRB 10-1580, IRB 10-1299), and immediately frozen for biomechanical characterization.

For the TE cartilage, MSCs were harvested using an explant technique, and passage 2 cells were chondroinduced as previously described (Dahl et al., 2011). Briefly, 4×10^5 isolated UC MSCs were placed in 0.3 mL of chondrogenic media in a 2-mL conical tube and grown for 21 days, after which the pellet was frozen. The conical shape of the tube causes cells to accumulate and create a pellet. Poly-L-lactic co-glycolytic acid (PLGA) nanofibers were electrospun into mats as previously described (Reed et al., 2009). The PLGA fabrication is performed via an electrospinning (electro static spraying) process. With the electrospinning method a liquid jet is formed via high voltage and small droplets, also solid fibers are formed through stretching of the solution and melting by electrostatic forces. Consequently, a mat is fabricated by collecting the nanofibers. 4×10^5 UC MSCs were seeded on the fibers and cultured in chondrogenic conditions for 14 days, then placed in a − 80 °C freezer (Quatela et al., 2006).

Human samples were cut into cylinders using a 2 mm punch biopsy, immediately following removal from ice. The samples were allowed to equilibrate at room temperature in a petri dish containing 1 × PBS at 4 °C. Images were obtained using a Leica EZ4D stereomicroscope at 16 × magnification. The diameter and height of the cylinders were measured using Leica LAS EZ software.

For the AFM testing: 3 conchal bowls, 3 costal cartilages, 3 microtia, 3 preauricular tags, 3 pellets, 3 PLGA mats and 6 unseeded mats were tested, while for the compression testing series: 5 microtia, 8 rib, 8 conchal bowl, 2 preauricular tags and 10 pellets samples were tested.

2.1. Atomic force microscopy (AFM) testing

Elastic moduli of the cartilage sample were measured using AFM (MFP-3DBio, Asylum Research, Santa Barbara, USA). TE samples (nanofiber scaffolds and pellets, both with and without chondroinduced UC MSCs), were immediately fixed to a glass slide, and submerged in PBS to maintain hydration. Samples remained hydrated and the AFM tip was submerged in PBS throughout the testing period. A silicon nitride with force constant of 1.75 N/m and a 25 μm spherical polystyrene tip (Novascan Technologies, Inc., Ames, IA) was used to indent