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Mechanical and biochemical mapping of human auricular cartilage for reliable assessment of tissue-engineered constructs

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ABSTRACT

It is key for successful auricular (AUR) cartilage tissue-engineering (TE) to ensure that the engineered cartilage mimics the mechanics of the native tissue. This study provides a spatial map of the mechanical and biochemical properties of human auricular cartilage, thus establishing a benchmark for the evaluation of functional competency in AUR cartilage TE.

Stress-relaxation indentation (instantaneous modulus, E_{in}; maximum stress, σ_{max}; equilibrium modulus, E_{eq}; relaxation half-life time, $t_{1/2}$; thickness, h) and biochemical parameters (content of DNA; sulfated-glycosaminoglycan, sGAG; hydroxyproline, HYP; elastin, ELN) of fresh human AUR cartilage were evaluated. Samples were categorized into age groups and according to their harvesting region in the human auricle (for AUR cartilage only).

AUR cartilage displayed significantly lower E_{in}, σ_{max}, E_{eq}, sGAG content; and significantly higher $t_{1/2}$, and DNA content than NAS cartilage. Large amounts of ELN were measured in AUR cartilage (> 15% ELN content per sample wet mass). No effect of gender was observed for either auricular or nasoseptal samples. For auricular samples, significant differences between age groups for h, sGAG and HYP, and significant regional variations for E_{in}, σ_{max}, E_{eq}, $t_{1/2}$, h, DNA and sGAG were measured. However, only low correlations between mechanical and biochemical parameters were seen ($R < 0.44$).

In conclusion, this study established the first comprehensive mechanical and biochemical map of human auricular cartilage. Regional variations in mechanical and biochemical properties were demonstrated in the auricle. This finding highlights the importance of focusing future research on efforts to produce cartilage grafts with spatially tunable mechanics.

1. Introduction

Surgical reconstruction with autologous cartilage or alloplastic implants is the only existing treatment for auricular defects. The current gold-standard technique – autologous ear reconstruction (Rotter et al., 2008) – is a multi-staged time-consuming procedure (Brent, 1992; Nagata, 1993), that ranks among the most complicated of reconstructive surgeries (Walton and Beahm, 2002). In short, autologous cartilage is harvested from the ribs, shaped appropriately and implanted subcutaneously. Auricular (AUR) cartilage tissue-engineering (TE) is a potential alternative that endeavors to circumvent the resulting donor-site morbidity by engineering rather than harvesting cartilage (Arevalo-Silva et al., 2000; Bichara et al., 2012; Cao et al., 1998; Cao et al., 1997; Haisch et al., 2002; Isogai et al., 2004, 2008; Kamil et al., 2004, 2003; Kusuhara et al., 2009; Liu et al., 2010; Neumeister et al., 2006; Ruszymah et al., 2011; Saim et al., 2000; Shieh et al., 2004; Xu et al., 2005).

Ideally tissue-engineered AUR cartilage should possess similar mechanical properties to the native tissue in order to withstand daily load (e.g. wearing spectacles, helmets, ear phones, etc.) and without causing discomfort (Nimeskern et al., 2014). Selecting autologous material for ear cartilage surgical reconstruction is difficult, where donations come from the nasal septum, auricle and rib. Whether the graft qualifies mechanically for surgical implantation is usually made

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from simple palpation. Mechanical properties of hyaline (e.g. nasoseptal, costal, articular cartilage) and fibrocartilage (e.g. intervertebral disk) have been extensively documented (Joshi et al., 1995; Mow and Guo, 2002; Rotter et al., 2002). The structure–function relationship linking composition and architecture to mechanical competency has been established for these cartilage subtypes (Humzah and Soames, 1988; Mow and Guo, 2002). The mechanical properties of AUR cartilage are, however, sparsely investigated (Naumann et al., 2002), and limited data are available for human cartilage (Nimeskern et al., 2014; Zopf et al., 2015). Unlike hyaline and fibrocartilage, AUR cartilage contains large amounts of elastin (ELN) fibers. Those fibers play a mechanical role in tissues such as arteries and skin (Debelle and Tamburro, 1999; Gosline et al., 2002), therefore the mechanical properties of AUR cartilage are expected to vary from other cartilage types (Nimeskern et al., 2014).

Mechanical evaluation has often been overlooked in AUR cartilage TE attempts. Many authors (Arevalo-Silva et al., 2000; Cao et al., 1998, 1997; Haisch et al., 2002; Isogai et al., 2004, 2008; Kamil et al., 2004, 2003; Kusuhara et al., 2009; Liu et al., 2010; Neumeister et al., 2006; Ruszymah et al., 2011; Saim et al., 2000; Shieh et al., 2004; Xu et al., 2005) report a qualitative mechanical assessment, while a few publications report quantitative data but without comparison to human AUR cartilage (Bichara et al., 2010; Britt and Park, 1998; Chang et al., 2003; Isogai et al., 2005; Ting et al., 1998). Indentation has been shown previously to be a good and sensitive first approximation for direct comparison between native and tissue-engineered constructs (Duda et al., 2000).

In light of this, the aim of this work is to establish a mechanical characterization of native human AUR cartilage in order to set a benchmark against which to evaluate TE constructs. Mechanical and biochemical properties of fresh AUR cartilage are determined and compared to hyaline nasoseptal (NAS) cartilage. Additionally spatial variation in mechanical properties, the influence of patient gender and age, and correlations between mechanical properties and biochemical composition are investigated.

2. Material and methods

2.1. Sample harvesting and preparation

Cadaveric auricles were harvested by Science Care (Phoenix, Arizona, USA, $n=4$) and Erasmus Medical Center (Rotterdam, the Netherlands, $n=11$) according to ethical guidelines of the respective institution. Additionally AUR and NAS cartilage was obtained from patients ($n=30$, AUR; $n=69$, NAS) undergoing middle-ear/cholesteatoma surgery (AUR) and functional septo- or septorhino-plasties (NAS) at University Hospital Zurich (Zurich, Switzerland), and Ulm University Medical Center (Ulm, Germany) according to the ethics regulations of the respective institution. AUR samples were harvested from 15 male and 12 female donors, NAS samples were harvested from 40 male and 12 female donors. Samples were pooled according to anthropomorphic age (Buikstra and Ubelaker, 1994) (child, <20 years; young adult, 20–34; middle adult, 35–49; and old adult, ≥ 50). All samples were shipped at 4 °C in phosphate buffered saline (PBS) supplemented with antibiotic/antimycotic (Gibco, Invitrogen Corporation, California, USA) to ETH Zurich (Zurich, Switzerland). The perichondrium was removed from AUR samples, and cylindrical plugs (5 mm, 1–2 mm thick) were cut perpendicular to the surface. Six harvesting regions were defined (anti-helix, AH; anti-tragus, AT; concha, CO; helix, HE; scapha, SC; tragus, TR), Fig. 1a. NAS plugs were similarly prepared, where samples originated from the center of the nasal septum, Fig. 1b. Differences in samples numbers for biomechanical and biochemical assays is due to sample loss during processing, unusual sample shape preventing mechanical analysis or limitations of biochemical assays.

2.2. Mechanical evaluation

Cartilage samples (AUR: $n=183$; NAS: $n=103$) were placed in close-fitting stainless steel cylindrical wells, and tested with a materials testing machine (Zwick Z005, Ulm, Germany) equipped with a 10 N load cell, built-in displacement control, and a cylindrical, plane-ended, stainless steel indenter (0.35 mm). During testing samples were immersed in PBS supplemented with antibiotic/antimycotic, and stress relaxation indentation tests were performed at room temperature, as described previously (Nimeskern et al., 2013; Stok et al., 2010). Briefly, a preload of 3 mN was applied to locate the sample surface and measure sample thickness, h, and held for 5 min. Five successive strain steps (5% of h per step) were applied, and specimens were left to relax for 20 min at each step. An in-house Matlab script converted force and displacement data to stress and strain, and instantaneous modulus (E_{in}), maximum stress (σ_{max}), equilibrium modulus (E_{eq}), relaxation half-life time ($t_{1/2}$) were determined. To estimate viscoelastic relaxation, $t_{1/2}$ is computed after the first strain application. It is defined as the time needed for stress to decrease from its maximum value halfway to its equilibrium value (Nimeskern et al., 2013).

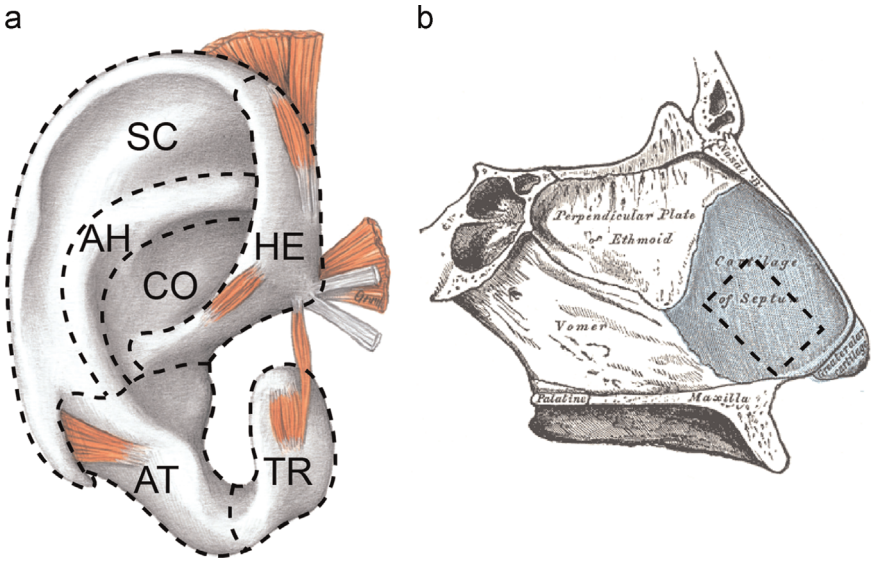

Fig. 1. (a) Map of the human auricle. Six harvesting regions are identified based on the ear morphology: anti-helix, AH, anti-tragus, AT, concha, CO, helix, HE, scapha, SC and tragus TR. Adapted from Atlas der Anatomie des Menschen, B.N. Tillmann, Springer–Lehrbuch (Tillmann, 2010). (b) Harvesting site for NAS cartilage. Adapted from Gray's Anatomy of the Human Body, Henry Gray (Gray, 1918).