Mechanical evaluation of bacterial nanocellulose as an implant material for ear cartilage replacement

Journal Article

Author(s):

Nimeskern, Luc; Martínez Ávila, Héctor; Sundberg, Johan; Gatenholm, Paul; [Müller, Ralph](#) ; Stok, Kathryn S.

Publication date:

2013-06

Permanent link:

<https://doi.org/10.3929/ethz-a-010576311>

Rights / license:

[In Copyright - Non-Commercial Use Permitted](#)

Originally published in:

Journal of the Mechanical Behavior of Biomedical Materials 22, <https://doi.org/10.1016/j.jmbbm.2013.03.005>

Mechanical integrity

Patient-specific biofabrication

- Bacterial nanocellulose (BNC) matches the mechanical moduli of human ear cartilage
- Different relaxation kinetics between BNC and native cartilage are observed
- Concepts for improved relaxation kinetics are proposed
- BNC can be produced in complex shapes, such as patient-specific auricles

Mechanical evaluation of bacterial nanocellulose as an implant material for ear cartilage replacement

Luc Nimeskern^a, Hector Martinez^b, Johan Sundberg^b, Paul Gatenholm^b, Ralph Müller^a, and Kathryn S. Stok^a

a: Institute for Biomechanics, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

b: Biopolymer Technology, Department of Chemical and Biological Engineering, Chalmers University of Technology, Arvid Wallgrens Backe 20, 41346 Göteborg, Sweden

Authors:

Luc Nimeskern

email: nluc@ethz.ch

Hector Martinez

email: hectorm@chalmers.se

Johan Sundberg

email: sundberj@chalmers.se

Paul Gatenholm

email: paul.gatenholm@chalmers.se

Ralph Müller

email: ram@ethz.ch

Kathryn S. Stok

email: kas@ethz.ch

Corresponding author:

Kathryn S. Stok, PhD.

Institute for Biomechanics, ETH Zurich

Wolfgang-Pauli-Strasse 10

8093 Zurich

Switzerland

Tel: +41 44 632 45 80

Fax: +41 44 633 15 73

Email: kas@ethz.ch

Number of words/characters:

abstract = 247

manuscript = 4725

Number Figures:

color = 2

b/w = 3

Abstract

Bacterial nanocellulose (BNC) is a novel non-degradable biocompatible material that promotes chondrocyte adhesion and proliferation. In this work, its potential use in ear cartilage tissue engineering (TE) is investigated. Firstly, the mechanical properties of native ear cartilage are measured in order to set a preliminary benchmark for ear cartilage replacement materials. Secondly, the capacity of BNC to match these requirements is assessed. Finally, a biofabrication process to produce patient-specific BNC auricular implants is demonstrated.

BNC samples ($n = 78$) with varying cellulose content (2.5 - 15%) were compared using stress-relaxation indentation with human ear cartilage ($n = 17$, from 4 males, aged 49 – 93 years old). Additionally, an auricle from a volunteer was scanned using a 3T MRI with a spoiled gradient-echo sequence. A negative ear mold was produced from the MRI data in order to investigate if an ear-shaped BNC prototype could be produced from this mold.

The results show that the instantaneous modulus E_{in} , equilibrium modulus E_{eq} , and maximum stress σ_{max} of the BNC samples are correlated to effective cellulose content. Despite significantly different relaxation kinetics, the E_{in} , E_{eq} and σ_{max} of BNC at 14% effective cellulose content reached values equivalent to ear cartilage (for E_{eq} , BNC: 2.4 ± 0.4 MPa and ear cartilage: 3.3 ± 1.3 MPa). Additionally, this work shows that BNC can be fabricated into patient-specific auricular shapes. In conclusion, BNC has the capability to reach mechanical properties of relevance for ear cartilage replacement, and can be produced in patient-specific ear shapes.

Keywords:

Stress relaxation, auricle, bacterial cellulose, microbial cellulose, tissue engineering.

1. Introduction

Bacterial nanocellulose (BNC) is a natural biopolymer produced by various species of bacteria, particularly *Gluconacetobacter xylinus* (formerly named *Acetobacter xylinus*) (Ross et al., 1991). It consists of an interconnected entangled network of cellulose fibrils similar in size to collagen fibers (approximately 30 nm wide) (Gatenholm and Klemm, 2010; Gelin et al., 2007) creating an extensive surface area holding a large amount of water (Czaja et al., 2007) while the whole structure is interconnected and stabilized by intra- and inter-fibrillar hydrogen bonds (O'Sullivan, 1997). This fibrillar structure provides high tensile mechanical characteristics to the material (Gea et al., 2011) and a hydrogel-like behavior as cellulose nanofibers bind water (Czaja et al., 2007). In contrast to plant cellulose, BNC is devoid of macromolecules such as lignin or hemicellulose (Petersen and Gatenholm, 2011). Additionally it is non-degradable in the human body, which lacks the enzymes to degrade cellulose, and it has been shown to be biocompatible (Backdahl et al., 2006; Helenius et al., 2006; Rosen et al., 2011); all factors which make BNC an exciting biomaterial for biomedical applications (Czaja et al., 2007; Petersen and Gatenholm, 2011).

Interconnected porosity is a key feature in a scaffold to allow the seeded chondrocytes to penetrate and migrate throughout the material. In its natural state, BNC is known to be impenetrable by cells, due to the small size of the pores (Backdahl et al., 2006; Klemm et al., 2005). However, this issue has been addressed by several investigators, who successfully synthesized BNC scaffolds with large pores, allowing the cells to penetrate the scaffold (Bäckdahl et al., 2008; Rambo et al., 2008). Therefore, in the scope of this work the mechanical

properties of BNC are the primary focus, including investigation of the tunability of the natural material.

Due to its good tensile mechanical properties, BNC has been used in previous work for blood vessel (Bodin et al., 2007a; Klemm et al., 2001; Malm et al., 2012), meniscus (Bodin et al., 2007b; Martinez et al., 2012), and articular cartilage tissue engineering (TE) strategies (Andersson et al., 2010; Svensson et al., 2005). It has been shown that BNC supports adhesion and proliferation of different cell types (Andersson et al., 2010; Backdahl et al., 2006; Bodin et al., 2010; Brackmann et al., 2011; Svensson et al., 2005), in particular chondrocytes (Andersson et al., 2010; Svensson et al., 2005). This ability to induce chondrocyte proliferation makes BNC a promising scaffold material for ear cartilage TE.

Although several groups have investigated engineering ear cartilage (Bichara et al., 2012), few successful outcomes have been reported (Yanaga et al., 2009). Many studies have investigated the potential of biodegradable scaffold materials (Cao et al., 1997; Haisch et al., 2002; Isogai et al., 2004; Kusuhara et al., 2009; Shieh et al., 2004); for example, results reported by Shieh et al. (Shieh et al., 2004) in an immuno-competent animal model showed poor shape stability due to an immune response presumably caused by degradation byproducts. As opposed to degradable biomaterials, non-degradable ones have the advantage of providing both durable mechanical properties and long-term chemical stability. Non-degradable biomaterials have been used for a wide range of biomedical applications (Chirila, 2001; Dziubla et al., 2001; Ruszymah et al., 2005; Xue and Greisler, 2003), but have only recently been investigated for ear cartilage engineering (Lee et al., 2011; Ruszymah et al., 2011; Zhou et al., 2011). Lee et al. (Lee et al., 2011) and

Ruszymah et al. (Ruszymah et al., 2011) investigated, *in vivo*, the use of commercial HDPE¹ implants covered with cell-fibrin constructs, and Zhou et al (Zhou et al., 2011) studied *in vivo* the potential of type I collagen implants reinforced with coiled titanium wires. Good histological (Lee et al., 2011; Ruszymah et al., 2011; Zhou et al., 2011) and mechanical results were reported (Lee et al., 2011). Nevertheless, the use of non-viscoelastic and stiff biomaterials such as HDPE or metals leads to complications - in particular, extrusion – reported with high occurrence in clinical outcomes for regular HDPE alloplastic implants (Cenzi et al., 2005). The use of a non-biodegradable, more compliant material such as BNC may provide an alternative implant material, on condition that the mechanical properties can be tuned to match the native tissue. That said, since a non-biodegradable implant like BNC will remain implanted over the patient life-time, it is critical that its mechanical properties are consistent with the native tissue, i.e. in this work, ear cartilage. Among the three types of cartilage present in the human body - hyaline cartilage, fibrocartilage and elastic cartilage - ear cartilage is classified as elastic cartilage (Sayed et al., 2010). The mechanical characteristics of hyaline cartilage (e.g. articular cartilage, nasoseptal cartilage) (Mow and Guo, 2002; Rotter et al., 2002) and fibrocartilage (e.g. meniscus) (Joshi et al., 1995) are well documented, yet there is little data available for ear cartilage (Naumann et al., 2002). In comparison to hyaline and fibrocartilage, elastic cartilage contains significantly more elastin (Naumann et al., 2002), which is known to be a highly resilient protein, and which plays a mechanically functional role in tissues like heart valves (Lee et al., 2001) and skin (Oxlund et al., 1988). For this reason, a profile of the mechanical properties of ear cartilage is required, since using published mechanical properties of hyaline cartilage or fibrocartilage may not be an appropriate substitute.

¹high density polyethylene

Two distinct mechanisms are involved in the response of cartilage to loading: the intrinsic mechanical properties of the ECM², and the resistance to interstitial fluid flow through the ECM (Mow and Guo, 2002), which is governed by the permeability and swelling pressure (Donnan osmotic pressure) of the ECM (Bartel DL et al., 2006). During loading, there is an instantaneous response involving a change in shape of the material without change in volume (instantaneous modulus); followed by a transient phase where the fibrillar stress relaxes and fluid flows out of the matrix (relaxation kinetic). Finally, the tissue reaches an equilibrium or steady-state response where fluid flow ceases; then the load is carried by the solid matrix, and is characterized by the elastic properties of only the solid component (equilibrium modulus) (Armstrong, 1986). Creep and stress relaxation tests are commonly performed to characterize the mechanical properties of cartilage to find these properties (Bartel DL et al., 2006).

Beside the control of the implant mechanics, it is key in the field of ear cartilage replacement, to control the external shape of the implant to obtain satisfying aesthetic results. This outcome is often used as a measure of success for a potential biomaterial (Lee et al., 2011; Shieh et al., 2004; Zhou et al., 2011). Most efforts in ear cartilage TE so far, have used standardized auricle shapes (Lee et al., 2011; Ruszymah et al., 2011; Shieh et al., 2004; Zhou et al., 2011), rather than patient-specific forms. Liu et al (Liu et al., 2010) describe a method to 3D print ear scaffolds using CT³ data from a patient scan. However, the use of CT is controversial since it uses a radiation source, and is not ideal for good quality soft tissue contrast; and hence the direct visualization of cartilage. Better soft tissue contrast would allow segmentation of the various soft tissues present, specifically ear cartilage, adipose and skin tissues, rather than the whole

² extracellular matrix

³ computed-tomography

ear. Reconstruction of the individual components would then further improve the aesthetic result. MRI⁴ is a non-invasive 3D imaging technique, which provides good soft tissue contrast. MRI is used clinically for evaluation of soft tissues such as articular cartilage (Gold et al., 2003), and is therefore proposed here as a promising imaging technique for ear cartilage. This study aims to assess whether BNC is a mechanically appropriate implant material for ear cartilage replacement. The objectives are firstly to measure the mechanical properties of native ear cartilage in order to define a preliminary benchmark for BNC implants, and secondly to assess whether BNC properties can be tuned to match these requirements. Finally, a biofabrication process to produce patient-specific BNC auricular implants is described.

2. Materials and methods

2.1. Ear cartilage harvesting

Human ear cartilage samples were obtained according to the ethics regulations of the University Hospital Zurich, UZH, (Zurich, Switzerland), the Ulm University Medical Center, UUMC, (Ulm, Germany) and the Erasmus Medical Center, EMC, (Rotterdam, The Netherlands). Samples were either harvested from patients undergoing ear reconstructive surgery (UZH, UUMC, and EMC) or from complete auricles obtained post mortem from human donors (EMC). The samples were shipped at 4°C in a phosphate buffer saline solution (PBS) supplemented with an antibiotic/antimycotic solution (Antibiotic/Antimycotic, Gibco®, Invitrogen Corporation, Carlsbad, California, USA) to ETH Zurich (Zurich, Switzerland). Upon arrival (~24 hours post-surgery), perichondrium was removed so as to expose the cartilage surface. Cylindrical plugs (Ø5 mm, ~1-2 mm thick) were then cut perpendicular to the cartilage surface. Stress relaxation

⁴magnetic resonance imaging

1
2
3
4 indentation was performed on each plug. A total of 17 ear cartilage plugs harvested from 4
5
6 males (49 -93 years old) were used for mechanical testing.
7
8
9

10 11 12 2.2. BNC synthesis 13

14 250 mL conical flasks (wide neck) were inoculated with *Gluconacetobacter xylinus* ATCC® 700178
15
16 (LGC Standards AB, Borås, Sweden). A 3 mL bacteria suspension and 300 mL of sterile culture
17
18 media (described by Matsuoka et al. (Matsuoka et al., 1996)) was added to each flask. The
19
20 conical flasks were placed in an incubator at 30°C. The bacterial culture continued until the
21
22 cellulose pellicle had reached a height of 5 cm, which took approximately 30 days. The protocol
23
24 used to remove the bacteria and its residues from the cellulose matrix was adapted from
25
26 previous studies (Helenius et al., 2006; Martinez et al., 2012). BNC pellicles were immersed in
27
28 0.1 M NaOH for 21 days, changing the solution every 24 hours, they were then rinsed with large
29
30 amounts of 60°C deionized water to remove bacterial residues and neutralize the pH.
31
32
33
34
35
36
37
38
39

40 2.3. Production of high cellulose content BNC pellicles 41

42 A sample was cut from each BNC pellicle and its initial cellulose content (CC_i) was determined
43
44 using a HB43 halogen moisture analyzer (Mettler-Toledo Inc., Columbus, OH, USA). The initial
45
46 cellulose content refers to the weight percent of cellulose produced by the bacteria, which is
47
48 approximately 1 wt%. This was computed using the equation:
49
50
51

$$52 \quad CC_i = (\text{Dry weight} / \text{Wet weight}) \times 100\%,$$

53
54 and is calculated directly after bacterial synthesis and cleaning. The BNC pellicles were then cut
55
56 to different initial heights H_i (equation 1). The cut pellicles were placed inside a stainless steel
57
58
59
60
61
62
63
64
65

1
2
3
4 tube ($\varnothing_i = 50$ mm) and a stainless steel rod was placed on top, and each pellicle was compressed
5
6
7 to a final height H_f of 1.00 ± 0.05 mm at a rate of 0.5 mm/min using an Instron 5565A (Instron,
8
9 Norwood, MA, USA). H_i is computed from equation 1, where CC_n is the desired nominal cellulose
10
11 content and H_f is the height after compression (nominally 1 mm):
12
13

$$H_i = H_f \cdot (CC_n / CC_i) \quad (1)$$

14
15
16
17 The pellicles were then stored at 4°C in vacuum-sealed bags.
18
19
20
21

22 *2.4. SEM characterization of BNC*

23
24 The nanocellulose fiber structure was characterized by scanning electron microscopy (SEM).
25
26 Cellulose samples of 1%, 5% and 10% nominal cellulose content ($n = 2$ for each cellulose
27
28 content) were quenched in liquid nitrogen, lyophilized for 72 hours (Heto PowerDry PL3000,
29
30 Thermo Fisher Scientific, Waltham, MA, USA), then mounted on SEM studs, sputtered with a
31
32 gold film and analyzed using a Leo Ultra 55 field emission gun (FEG) SEM (Carl Zeiss AG,
33
34 Oberkochen, Germany). Images were taken at a magnification of 25000x.
35
36
37
38
39
40
41
42

43 *2.5. BNC sample preparation for mechanical testing*

44
45 For mechanical testing, cylindrical plugs ($\varnothing 5$ mm, ~1 mm thick) were cut with a biopsy punch
46
47 from BNC pellicles of 2.5%, 5%, 7.5%, 10%, 12.5% and 15% nominal cellulose content ($n = 13$ for
48
49 each cellulose content, $n_{\text{total}} = 78$). In order to determine the effective cellulose content, the
50
51 plugs were weighed before mechanical testing. After mechanical testing, the samples were
52
53 freeze-dried and the dry weight was measured. The effective cellulose content (CC_e) refers to
54
55
56
57
58
59
60
61
62
63
64
65

the true cellulose content of the plugs during testing and was computed according to equation

2.

$$CC_e = (\text{Dry weight after testing} / \text{Wet weight before testing}) \times 100\% \quad (2)$$

2.6. Mechanical testing

Auricular cartilage ($n = 17$) and BNC samples ($n_{\text{total}} = 78$) with varying cellulose content were placed in close-fitting stainless steel cylindrical wells. Mechanical testing was performed with a materials testing machine (Zwick Z005, Ulm, Germany) equipped with a 10 N load cell, a built-in displacement control, and a cylindrical, plane ended, stainless steel indenter ($\varnothing 0.35$ mm). During mechanical testing the cartilage samples were immersed in PBS supplemented with antibiotic/antimycotic solution, BNC samples were immersed in Millipore™ water. Stress relaxation testing was performed as described previously (Stok et al., 2010). In short, a preload of 3 mN was first applied on the sample to locate the sample surface and measure sample thickness, and held for 5 minutes. Five successive strain steps were then applied in 5% increments of the original sample thickness, and specimens were left to relax for 1 hour (BNC) and 20 minutes (cartilage) at each step. The hold time was defined as the time necessary to reach equilibrium. An in-house Matlab® script was used to convert force and displacement data to stress and strain. Measurements of maximum stress σ_{max} , equilibrium modulus E_{eq} , and instantaneous modulus E_{in} were determined for every sample, as described previously (Stok et al., 2010). Additionally, a relaxation half-life time $t_{1/2}$ and a characteristic relaxation time τ were computed to estimate the viscoelastic relaxation after the first strain application. $t_{1/2}$ was defined as the time needed for the stress to decrease to half of its maximum value, and τ was

obtained by fitting an exponential decay function to the stress-time data (see Figure 1). In equation 3, A and τ are the two fitted parameters, σ_i is the stress immediately following the first strain application, σ_{eq} is the equilibrium stress at the end of the first strain step and T is the hold time. Note that by definition $f(0)=\sigma_i$ and $f(T) \approx \sigma_{eq}$.

$$f(t) = (\sigma_i - A) + t / T \cdot (A + \sigma_{eq} - \sigma_i) + A \cdot \exp(-t/\tau) \quad (3)$$

2.7. MRI of human auricles for the biofabrication of patient-specific BNC implants

An MRI scan of the external ear was performed on a male 28 year-old volunteer with a Magnetom Skyra 3T MRI (Siemens AG, Erlangen, Germany). The volunteer gave informed consent for the study. A spoiled gradient-echo sequence (T1 VIBE) with fat saturation was used to scan a $5.76 \times 5.76 \times 5.64 \text{ cm}^3$ VOI⁵ centered on the auricle. The XY plane was chosen parallel to the sagittal plane. An in-plane (XY) resolution of $0.45 \text{ mm} \times 0.45 \text{ mm}$ and an out-of-plane (Z) resolution of 0.40 mm were used. Manual segmentation of the external ear shape was performed, and a 3D surface mesh was created and saved in STereoLithography (STL) file format (suitable for rapid prototyping). A nylon ear replica was produced by Materialise (Leuven, Belgium) from the 3D surface mesh using selective laser sintering. A negative ear mold was produced by casting silicone around the ear model. The negative silicone mold consisted of three pieces for easy retrieval of the ear model and, subsequently, the 3D ear-shaped BNC implant prototype.

2.8. 3D biofabrication system

⁵volume of interest

A bioprinter, developed in-house and consisting of a high precision micro-dispensing system - used to dispense controlled volumes of bacterial culture media, and a precision motion system - used for controlling the dispensing location, was used for the biofabrication of a patient-specific ear-shaped BNC implant prototype (see figure 2). The motion system consists of a two-axis linear stepper motor gantry stage (H2W Technologies Inc, Valencia, CA, USA) connected to a NI MID-7604 stepper power drive (National Instruments, Austin, TX, USA), see figure 2a,c, which provides stepper motor control from a NI PCI-7344 controller (National Instruments, Austin, TX, USA). NI Motion Assistant 2.7 (National Instruments, Austin, TX, USA) was used to program the controller for motion control (figure 2c). The micro-dispensing system (figure 2b) consists of an M/2 Very High Speed micro-dispensing valve (The LEE company, Westbrook, CT, USA) and a Spike and Hold Driver (The LEE company, Westbrook, CT, USA) connected to the controller and programmed in NI Motion Assistant. The micro-dispensing valve coupled to an atomizing nozzle (The LEE company), was connected to a pressurized tank (Model 66839, Central Pneumatic) containing sterile culture media (figure 2a). The pressure inside the tank was monitored using a Motorola MPX4250AP absolute pressure sensor (Freescale Semiconductor Inc, Austin, TX, USA) connected to a NI USB-6009 data acquisition device (National Instruments, Austin, TX, USA). The pressure was monitored and recorded using NI LabVIEW Signal Express 2009 (National Instruments, Austin, TX, USA). A silicone rubber heater connected to an EHG® SL10 process controller (Watlow Electric Manufacturing Company, Columbia, MO, USA) was used to maintain culture media temperature at 30°C. The micro-dispensing valve and gantry stage were placed inside a laminar airflow bench during bacterial culture. The pressure tank, silicone tubing,

fittings and atomizing nozzle were steam sterilized (1 bar, 121°C) for 20 min. The VHS micro-dispensing valve was sterilized by flushing it repeatedly with 70% ethanol.

2.9. Synthesis of patient-specific ear-shaped BNC implant prototype

The negative silicone mold was inoculated with 1 ml of bacteria suspension and positioned in the bioprinter. After 24 hours, a thin film of BNC had grown. Thereafter, the micro-dispensing valve was programmed to spray 500 µL of sterile culture media into the mold every 6 hours and the motion system was programmed to move along the helix of the mold during dispensing. The bacterial culture continued for 18 days until the cellulose layer filled the silicone mold. The ear-shaped BNC implant prototype was removed from the mold and cleaned following the same protocol described for the BNC pellicles.

2.10. Statistical analysis

All statistical analyses were performed with R (version 2.13.0, R Foundation for Statistical Computing, Vienna, Austria). A univariate analysis of variance was also used to test significant differences ($p < 0.05$) in the measured mechanical parameters between BNC and ear cartilage. The relationships between effective cellulose content and the measured mechanical parameters were evaluated using Pearson's linear regression coefficient of correlation, R . All results are displayed as mean $\pm$ standard deviation (SD).

3. Results

3.1. Mechanical testing of ear cartilage

The results obtained from the stress relaxation testing show an expected viscoelastic behavior of human ear cartilage (see figure 1b). After processing the data from the stress-strain and stress-time curves, the following measurements were determined; 6.4 ± 3.2 MPa for E_{in} , 3.3 ± 1.3 MPa for E_{eq} , 1.6 ± 0.5 MPa for σ_{max} , 2.1 ± 1.3 s for $t_{1/2}$ and 6.0 ± 2.6 s for τ (Figure 4).

3.2. Production and mechanical testing of high cellulose content BNC

BNC pellicles with effective cellulose content ranging from 4.1% up to 13.7% were produced. The network structure of native (1% cellulose content) and compressed (5% and 10%) BNC was characterized by SEM. As seen in figure 3, the spacing between the nanocellulose fibers decreases with increasing cellulose concentration. Compressive properties, E_{in} , E_{eq} , and σ_{max} , were correlated to effective cellulose content with R values of 0.66, 0.79, and 0.78, respectively ($p < 0.05$ in all cases), see figure 4. The E_{eq} of BNC could be tuned to values around 3.2 MPa at the highest effective cellulose content (13.7%). Despite observing a typical stress relaxation behavior in all BNC samples, the relaxation half-life, $t_{1/2}$, of BNC (0.4 ± 0.1 s) was significantly less than ear cartilage (2.1 ± 1.3 s; $p < 0.05$). The same observation was made for τ (3.7 ± 1.0 s and 6.0 ± 2.6 s, respectively; $p < 0.05$).

3.3. MRI of human auricles for the biofabrication of patient-specific BNC implants

The ear cartilage and external ear volume of a volunteer was successfully segmented and reconstructed from the MR image data (Figure 5a-b). A negative silicone mold was used to guide the bacteria during bacterial culture to reproduce the large-scale features of the outer ear; a process taking about 18 days (Figure 5c). Subsequently, a 3D patient-specific BNC implant

prototype (1% effective cellulose content), mimicking the external ear shape, was successfully synthesized using this mold in a novel biofabrication process (figure 5d).

4. Discussion

In this study, values for E_{in} , E_{eq} , σ_{max} , $t_{1/2}$ and τ of ear cartilage were measured using stress-relaxation indentation. These values represent a preliminary benchmark for BNC implants aimed at ear cartilage TE. Moderate standard deviations are observed. These variations are expected to result from factors such as donor age, gender and harvesting location within the auricle. A detailed investigation of these factors is out of the scope of the present study and will be performed in future work, in order to provide a standard for ear cartilage replacement strategies. The observed variability of cartilage mechanical properties emphasizes the need of an ear cartilage replacement material with spatially tunable mechanical properties.

The compressive mechanical properties of BNC were demonstrated to be tunable and correlated to effective cellulose content. The computed correlation coefficients ($R = 0.66, 0.79, 0.78$ for E_{in} , E_{eq} , σ_{max} , respectively) are moderate. This is likely due to an imperfect reproducibility of the cellulose content measurement protocol. Nonetheless, by varying effective cellulose content in BNC, an increase in E_{eq} from 0.06 to 3.2 MPa was observed; partly covering the range of values observed for ear cartilage (3.3 ± 1.3 MPa, see figure 4). The same observation holds for E_{in} and σ_{max} . These parameters of tunability make BNC a promising candidate as a non-biodegradable implant material for ear cartilage. Firstly, a further increase in E_{in} , E_{eq} , and σ_{max} of BNC could be achieved through escalation of the cellulose content, so as to cover the whole range of equilibrium modulus observed in ear cartilage. In future, implants with

1
2
3
4 varying local mechanical properties could be produced to more closely match the local
5
6 mechanical properties of the native tissue, as explained above.
7
8

9 Although similar equilibrium moduli were measured for BNC and ear cartilage (see figure 4), the
10 relaxation measures - namely $t_{1/2}$ (0.4 ± 0.1 s and 2.1 ± 1.3 s, respectively) and τ (3.7 ± 1.0 s and
11
12 6.0 ± 2.6 s, respectively), were significantly shorter for BNC. As opposed to BNC which is
13
14 electrically neutral, cartilage contains charged groups by way of the GAG⁶ content trapped in its
15
16 collagen matrix (Mow and Guo, 2002). These charged groups play a role in the relaxation
17
18 behavior of cartilage, via charge-dependent osmotic swelling pressures (i.e. Donnan osmotic
19
20 pressure) enhanced during compression (Mow and Guo, 2002). Therefore, the introduction of
21
22 electrical charge into the BNC network could provide mechanical properties that better mimic
23
24 those of native cartilage, in particular improved relaxation kinetics (higher relaxation half time
25
26 and characteristic relaxation time). In chondrocyte-seeded BNC constructs this introduction of
27
28 electrical charges could be achieved by the GAG produced by the seeded cells; which would
29
30 require adequate seeding and distribution throughout the scaffold. Alternatively this could be
31
32 obtained by chemical modification of BNC. Numerous chemical modifications of BNC have been
33
34 reported in literature, aimed at promoting cell adhesion on BNC (Bodin et al., 2007a; Kalaskar et
35
36 al., 2008), functionalizing it with drugs (Barud et al., 2008; Klechkovskaya et al., 2008; Nguyen et
37
38 al., 2008) or tuning its biodegradability (Kusuhara et al., 2009) and producing nanocomposites
39
40 (Grande et al., 2009; Wan et al., 2007). Interestingly Svensson et al. (Svensson et al., 2005)
41
42 demonstrated that chemical modifications such as sulfonation or phosphorylation could also
43
44 increase BNC compressive modulus (i.e. E_{in}), thus artificially mimicking the presence of charged
45
46 GAG in the BNC matrix.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

61 ⁶glycosaminoglycan
62
63
64
65

1
2
3
4 In addition to appropriate mechanical behavior, penetration and proliferation of seeded
5
6 chondrocytes throughout the material is a key aspect for the use of BNC as a scaffold material.
7
8 Native BNC is known to be impenetrable to cells (Backdahl et al., 2006; Klemm et al., 2005). To
9
10 circumvent this issue, Bäckdahl et al. (Backdahl et al., 2006) synthesized porous BNC scaffolds by
11
12 introducing porogens as space holders in the culture medium, and subsequently removing
13
14 them; leaving empty pores in the BNC network. They reported tunable pore size and pore
15
16 interconnectivity by controlling the porogen size and density, as well as better ingrowth of cells
17
18 in comparison to similar studies on non-porous BNC pellicles. However, the tensile mechanical
19
20 properties of these porous BNC scaffolds were drastically reduced (~5 MPa in native BNC
21
22 compared to ~2 MPa in porous BNC) (Bodin et al., 2010). In future work, the approach
23
24 developed by Bäckdahl et al. (Bäckdahl et al., 2008) will be combined with the one presented in
25
26 this study, in order to create bilayer BNC scaffolds. These scaffolds will contain a high cellulose
27
28 content BNC layer, tuned to the specific application, and a porous layer to support the cells and
29
30 enhance ECM formation. As chondrocytes have been reported to proliferate in native BNC
31
32 (Andersson et al., 2010; Svensson et al., 2005), these scaffolds will provide an adequate
33
34 environment for chondrocyte proliferation in the porous region, as well as mechanical stability
35
36 throughout the high cellulose content layer.
37
38 Besides the control of the implant porosity, it is key in the field of ear cartilage replacement, to
39
40 control the external shape of the implant to obtain satisfying aesthetic results. Several
41
42 investigators reported the production of BNC scaffolds with customized shapes (Bäckdahl et al.,
43
44 2008; Bodin et al., 2007b; Czaja et al., 2004). For example, a tube-like cultivation bioreactor with
45
46 oxygen-permeable walls has been developed to create hollow BNC tubes for use in blood vessel
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 replacement (Bäckdahl et al., 2008; Klemm et al., 2001). The 3D BNC implant prototype (1%
2
3
4 cellulose content) in the shape of a human auricle, presented in this study, demonstrates the
5
6
7 ability of BNC to be produced in more complex 3D shapes. This was made possible through the
8
9
10 use of a bottom-up biofabrication approach, which requires a mold to guide the bacteria to
11
12
13 reproduce the large-scale features of the tissue of interest (figure 5). Most efforts in ear
14
15
16 cartilage TE have used standardized auricle shapes (Lee et al., 2011; Ruszymah et al., 2011;
17
18
19 Shieh et al., 2004; Zhou et al., 2011). The BNC implant prototype presented in this study was
20
21
22 obtained from patient-specific MR image data which produces a result compatible with patient
23
24
25 expectations. In order to match the mechanical properties of ear cartilage as measured in this
26
27
28 study, one can scale-up the dimensions of the mold in the z-direction. This would allow the
29
30
31 bacteria to synthesize enlarged ear-shaped BNC implants, which can then be compressed to the
32
33
34 original ear size, and desired cellulose content. An alternate method for the creation of patient-
35
36
37 specific ear scaffolds was presented by Liu et al. (Liu et al., 2010) who described the 3D printing
38
39
40 of an ear scaffold using computed-tomography data. The advantages of using MRI are, firstly the
41
42
43 absence of potential radiation damage to the patient, and secondly a better soft tissue contrast
44
45
46 and hence the direct visualization of cartilage (figure 5). This makes it possible to produce BNC
47
48
49 implants which mimic the shape of ear cartilage, rather than the whole ear.
50
51
52 An important caveat however, is the time required to produce patient-specific ear-shaped BNC
53
54
55 implants, which is significantly long. One alternative would be to produce the ear-shaped BNC
56
57
58 implants by compressing BNC pellicles in an ear mold to shape the pellicle into an ear. Once the
59
60
61 pellicle is molded to the final shape, it can then be freeze-dried to physically crosslink the BNC
62
63
64 network. This method of producing ear-shaped BNC implants will significantly shorten the
65

production time, as the BNC pellicles can be pre-produced and kept as an off the shelf product, which can then be compressed to the patient's ear shape. This way the BNC production will not cause a delay in the production process of the implant.

Consequently, future work will aim at producing 3D ear scaffolds mimicking the shape of ear cartilage only. These scaffolds will consist of a high cellulose content core surrounded by a porous layer, so as to combine mechanical stability and porosity to allow chondrocytes to proliferate throughout the porous part and synthesize ear cartilage matrix.

5. Conclusion

This study aimed to assess whether BNC is a mechanically appropriate implant material for ear cartilage replacement. Firstly, mechanical properties of native ear cartilage were measured in order to define a preliminary benchmark required for BNC ear cartilage replacement implants. Secondly, the compressive mechanical properties of BNC materials could be tuned to match the mechanical requirements of native human ear cartilage by increasing the effective cellulose content. The difference in relaxation kinetics is likely due to the lack of charge-dependent osmotic swelling pressure in the BNC construct, as opposed to the native ECM. This important property of fluid flow resistance in the BNC network may be improved by chemical modification or even after implantation; where a native ECM would be produced by the cells in the porous layer of the BNC scaffold. Additionally the ability of the material to be produced in patient-specific and complex 3D shapes, such as a human auricle, was demonstrated. Ongoing work focuses on further enhancing BNC mechanics, and on developing BNC scaffolds surrounded by a porous layer to provide adequate environment for chondrocyte proliferation. In conclusion, BNC

shows promise for engineering non-biodegradable ear implants with suitable mechanical properties and patient-specific shapes.

6. Funding sources:

This study was supported by the Swiss National Science Foundation (NRP63) and ERA-NET/EuroNanoMed (EAREG-406340-131009/1).

7. Acknowledgements

Nicole Rotter and Stefan Klein at the Ulm University Medical Center, Ulm, Germany for MRI support and cartilage sample supply, Gerjo van Osch and Mieke Pleumeekers at the Erasmus MC, Rotterdam, The Netherlands for cartilage sample supply, and David Holzmann at the University Hospital Zurich, Zurich, Switzerland for cartilage sample supply.

9. Conflict of interest

All authors have no conflict of interest.

10. References

- Andersson, J., Stenhamre, H., Bäckdahl, H., Gatenholm, P., 2010. Behavior of human articular chondrocytes on microporous bacterial cellulose scaffolds. J Biomed Mat Res A 94, 1124-1132.
- Armstrong, C.G., 1986. An analysis of the stresses in a thin layer of articular cartilage in a synovial joint. Eng Med 15, 55-61.

1
2
3
4 Bäckdahl, H., Esguerra, M., Delbro, D., Risberg, B., Gatenholm, P., 2008. Engineering
5
6 microporosity in bacterial cellulose scaffolds. J Tissue Eng Regen Med 2, 320-330.
7
8
9
10 Backdahl, H., Helenius, G., Bodin, A., Nannmark, U., Johansson, B.R., Risberg, B., Gatenholm, P.,
11
12 2006. Mechanical properties of bacterial cellulose and interactions with smooth muscle cells.
13
14 Biomaterials 27, 2141-2149.
15
16
17
18 Bartel DL, Davy DT, Keaveny TM, 2006. Orthopaedic biomechanics: mechanics and design in
19
20 musculoskeletal systems. Pearson Prentice Hall, Upper Saddle River, New Jersey.
21
22
23
24 Barud, H.S., Barrios, C., Regiani, T., Marques, R.F.C., Verelst, M., Dexpert-Ghys, J., Messaddeq,
25
26 Y., Ribeiro, S.J.L., 2008. Self-supported silver nanoparticles containing bacterial cellulose
27
28 membranes. Mater Sci Eng C Mater Biol Appl 28, 515-518.
29
30
31
32
33 Bichara, D.A., O'Sullivan, N.A., Pomerantseva, I., Zhao, X., Sundback, C.A., Vacanti, J.P.,
34
35 Randolph, M.A., 2012. The tissue-engineered auricle: past, present, and future. Tissue Eng Part
36
37 B Rev 18, 51-61.
38
39
40
41
42 Bodin, A., Ahrenstedt, L., Fink, H., Brumer, H., Risberg, B., Gatenholm, P., 2007a. Modification of
43
44 Nanocellulose with a Xyloglucan–RGD Conjugate Enhances Adhesion and Proliferation of
45
46 Endothelial Cells: Implications for Tissue Engineering. Biomacromolecules 8, 3697-3704.
47
48
49
50
51 Bodin, A., Bharadwaj, S., Wu, S., Gatenholm, P., Atala, A., Zhang, Y., 2010. Tissue-engineered
52
53 conduit using urine-derived stem cells seeded bacterial cellulose polymer in urinary
54
55 reconstruction and diversion. Biomaterials 31, 8889-8901.
56
57
58
59
60
61
62
63
64
65

1
2
3
4 Bodin, A., Concaro, S., Brittberg, M., Gatenholm, P., 2007b. Bacterial cellulose as a potential
5
6
7 meniscus implant. *J Tissue Eng Regen Med* 1, 406-408.
8
9
10 Brackmann, C., Esguerra, M., Olausson, D., Delbro, D., Krettek, A., Gatenholm, P., Enejder, A.,
11
12
13 2011. Coherent anti-Stokes Raman scattering microscopy of human smooth muscle cells in
14
15 bioengineered tissue scaffolds. *J Biomed Opt* 16, 021115.
16
17
18 Cao, Y., Vacanti, J.P., Paige, K.T., Upton, J., Vacanti, C.A., 1997. Transplantation of chondrocytes
19
20
21 utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a
22
23
24 human ear. *Plast Reconstr Surg* 100, 297-302.
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Cenzi, R., Farina, A., Zuccarino, L., Carinci, F., 2005. Clinical outcome of 285 Medpor grafts used for craniofacial reconstruction. *J Craniofac Surg* 16, 526-530.

Chirila, T.V., 2001. An overview of the development of artificial corneas with porous skirts and the use of PHEMA for such an application. *Biomaterials* 22, 3311-3317.

Czaja, W., Romanovicz, D., Brown, R.m., 2004. Structural investigations of microbial cellulose produced in stationary and agitated culture. *Cellulose* 11, 403-411.

Czaja, W.K., Young, D.J., Kawecki, M., Brown, R.M., Jr., 2007. The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules* 8, 1-12.

Dziubla, T.D., Torjman, M.C., Joseph, J.I., Murphy-Tatum, M., Lowman, A.M., 2001. Evaluation of porous networks of poly(2-hydroxyethyl methacrylate) as interfacial drug delivery devices. *Biomaterials* 22, 2893-2899.

Gatenholm, P., Klemm, D., 2010. Bacterial nanocellulose as a renewable material for biomedical applications. *MRS Bulletin* 35, 208-213.

Gea, S., Reynolds, C.T., Roohpour, N., Wirjosentono, B., Soykeabkaew, N., Bilotti, E., Peijs, T., 2011. Investigation into the structural, morphological, mechanical and thermal behaviour of bacterial cellulose after a two-step purification process. *Bioresour Technol* 102, 9105-9110.

Gelin, K., Bodin, A., Gatenholm, P., Mihranyan, A., Edwards, K., Strømme, M., 2007. Characterisation of water in bacterial cellulose using dielectric spectroscopy and electron microscopy. *Polymer* 48, 7623-7631.

Gold, G.E., McCauley, T.R., Gray, M.L., Disler, D.G., 2003. What's new in cartilage? *Radiographics* 23, 1227-1242.

Grande, C.J., Torres, F.G., Gomez, C.M., Carmen Bañó, M., 2009. Nanocomposites of bacterial cellulose/hydroxyapatite for biomedical applications. *Acta biomaterialia* 5, 1605-1615.

Haisch, A., Klaring, S., Groger, A., Gebert, C., Sittinger, M., 2002. A tissue-engineering model for the manufacture of auricular-shaped cartilage implants. *Eur Arch Otorhinolaryngol* 259, 316-321.

Helenius, G., Backdahl, H., Bodin, A., Nannmark, U., Gatenholm, P., Risberg, B., 2006. In vivo biocompatibility of bacterial cellulose. *J Biomed Mater Res A* 76, 431-438.

Isogai, N., Asamura, S., Higashi, T., Ikada, Y., Morita, S., Hillyer, J., Jacquet, R., Landis, W.J., 2004. Tissue engineering of an auricular cartilage model utilizing cultured chondrocyte-poly(L-lactide-epsilon-caprolactone) scaffolds. *Tissue Eng* 10, 673-687.

Joshi, M.D., Suh, J.-K., Marui, T., Woo, S.L.Y., 1995. Interspecies variation of compressive biomechanical properties of the meniscus. *Journal of biomedical materials research* 29, 823-828.

Kalaskar, D.M., Gough, J.E., Ulijn, R.V., Sampson, W.W., Scurr, D.J., Rutten, F.J., Alexander, M.R., Merry, C.L.R., Eichhorn, S.J., 2008. Controlling cell morphology on amino acid-modified cellulose. *Soft Matter* 4, 1059-1065.

Klechkovskaya, V.V., Volkov, V.V., Shtykova, E.V., Arkharova, N.A., Baklagina, Y.G., Khripunov, A.K., Smyslov, R.Y., Borovikova, L.N., Tkachenko, A.A., 2008. Network model of acetobacter xylinum cellulose intercalated by drug nanoparticles nanomaterials for application in medicine and biology, in: Giersig, M., Khomutov, G.B. (Eds.), *Nanomaterials for application in medicine and biology*. Springer Netherlands, pp. 165-177.

Klemm, D., Heublein, B., Fink, H.-P., Bohn, A., 2005. Cellulose: fascinating biopolymer and sustainable raw material. *Angew Chem Int Ed Engl* 44, 3358-3393.

Klemm, D., Schumann, D., Udhardt, U., Marsch, S., 2001. Bacterial synthesized cellulose - artificial blood vessels for microsurgery. *Prog Polym Sci* 26, 1561-1603.

Kusuhara, H., Isogai, N., Enjo, M., Otani, H., Ikada, Y., Jacquet, R., Lowder, E., Landis, W.J., 2009. Tissue engineering a model for the human ear: assessment of size, shape, morphology, and gene expression following seeding of different chondrocytes. *Wound Repair Regen* 17, 136-146.

Lee, S.J., Broda, C., Atala, A., Yoo, J.J., 2011. Engineered cartilage covered ear implants for auricular cartilage reconstruction. *Biomacromolecules* 12, 306-313.

Lee, T.C., Midura, R.J., Hascall, V.C., Vesely, I., 2001. The effect of elastin damage on the mechanics of the aortic valve. *Journal of biomechanics* 34, 203-210.

Liu, Y., Zhang, L., Zhou, G., Li, Q., Liu, W., Yu, Z., Luo, X., Jiang, T., Zhang, W., Cao, Y., 2010. In vitro engineering of human ear-shaped cartilage assisted with CAD/CAM technology. *Biomaterials* 31, 2176-2183.

Malm, C.J., Risberg, B., Bodin, A., Backdahl, H., Johansson, B.R., Gatenholm, P., Jeppsson, A., 2012. Small calibre biosynthetic bacterial cellulose blood vessels: 13-months patency in a sheep model. *Scand Cardiovasc J* 46, 57-62.

Martinez, H., Brackmann, C., Enejder, A., Gatenholm, P., 2012. Mechanical stimulation of fibroblasts in micro-channeled bacterial cellulose scaffolds enhances production of oriented collagen fibers. *J Biomed Mat Res A* 100, 948-957.

Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O., Yoshinaga, F., 1996. A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* subsp *sucrofermentans*. *Biosci Biotechnol Biochem* 60, 575-579.

Mow, V.C., Guo, X.E., 2002. Mechano-electrochemical properties of articular cartilage: their inhomogeneities and anisotropies. *Annu Rev Biomed Eng* 4, 175-209.

Naumann, A., Dennis, J.E., Awadallah, A., Carrino, D.A., Mansour, J.M., Kastenbauer, E., Caplan, A.I., 2002. Immunochemical and mechanical characterization of cartilage subtypes in rabbit. *J Histochem Cytochem* 50, 1049-1058.

Nguyen, V.T., Gidley, M.J., Dykes, G.A., 2008. Potential of a nisin-containing bacterial cellulose film to inhibit *Listeria monocytogenes* on processed meats. *Food Microbiology* 25, 471-478.

O'Sullivan, A., 1997. Cellulose: the structure slowly unravels. *Cellulose* 4, 173-207.

Oxlund, H., Manschot, J., Viidik, A., 1988. The role of elastin in the mechanical properties of skin. *Journal of biomechanics* 21, 213-218.

Petersen, N., Gatenholm, P., 2011. Bacterial cellulose-based materials and medical devices: current state and perspectives. *Appl Microbiol Biotechnol* 91, 1277-1286.

Rambo, C.R., Recouvreux, D.O.S., Carminatti, C.A., Pitlovanciv, A.K., Antônio, R.V., Porto, L.M., 2008. Template assisted synthesis of porous nanofibrous cellulose membranes for tissue engineering. *Mater Sci Eng C Mater Biol Appl* 28, 549-554.

Rosen, C.L., Steinberg, G.K., DeMonte, F., Delashaw, J.B., Jr., Lewis, S.B., Shaffrey, M.E., Aziz, K., Hantel, J., Marciano, F.F., 2011. Results of the prospective, randomized, multicenter clinical trial evaluating a biosynthesized cellulose graft for repair of dural defects. *Neurosurgery* 69, 1093-1103.

Ross, P., Mayer, R., Benziman, M., 1991. Cellulose biosynthesis and function in bacteria. *Microbiol Rev* 55, 35-58.

Rotter, N., Bonassar, L.J., Tobias, G., Lebl, M., Roy, A.K., Vacanti, C.A., 2002. Age dependence of biochemical and biomechanical properties of tissue-engineered human septal cartilage. *Biomaterials* 23, 3087-3094.

Ruszymah, B.H., Chua, K., Latif, M.A., Hussein, F.N., Saim, A.B., 2005. Formation of in vivo tissue engineered human hyaline cartilage in the shape of a trachea with internal support. *Int J Pediatr Otorhinolaryngol* 69, 1489-1495.

Ruszymah, B.H., Chua, K.H., Mazlyzam, A.L., Aminuddin, B.S., 2011. Formation of tissue engineered composite construct of cartilage and skin using high density polyethylene as inner scaffold in the shape of human helix. *Int J Pediatr Otorhinolaryngol* 75, 805-810.

Sayed, K.E., Haisch, A., John, T., Marzahn, U., Lohan, A., Müller, R.D., Kohl, B., Ertel, W., Stoelzel, K., Schulze-Tanzil, G., 2010. Heterotopic autologous chondrocyte transplantation—A realistic approach to support articular cartilage repair? *Tissue engineering. Part B, Reviews* 16, 603-616.

Shieh, S.-J., Terada, S., Vacanti, J.P., 2004. Tissue engineering auricular reconstruction: in vitro and in vivo studies. *Biomaterials* 25, 1545-1557.

Stok, K.S., Lisignoli, G., Cristino, S., Facchini, A., Muller, R., 2010. Mechano-functional assessment of human mesenchymal stem cells grown in three-dimensional hyaluronan-based scaffolds for cartilage tissue engineering. *J Biomed Mater Res A* 93, 37-45.

Svensson, A., Nicklasson, E., Harrah, T., Panilaitis, B., Kaplan, D.L., Brittberg, M., Gatenholm, P., 2005. Bacterial cellulose as a potential scaffold for tissue engineering of cartilage. *Biomaterials* 26, 419-431.

Wan, Y.Z., Huang, Y., Yuan, C.D., Raman, S., Zhu, Y., Jiang, H.J., He, F., Gao, C., 2007. Biomimetic synthesis of hydroxyapatite/bacterial cellulose nanocomposites for biomedical applications. *Mater Sci Eng C Mater Biol Appl* 27, 855-864.

1
2
3
4 Xue, L., Greisler, H.P., 2003. Biomaterials in the development and future of vascular grafts. J
5
6 Vasc Surg 37, 472-480.
7
8
9

10 Yanaga, H., Imai, K., Fujimoto, T., Yanaga, K., 2009. Generating ears from cultured autologous
11
12 auricular chondrocytes by using two-stage implantation in treatment of microtia. Plast Reconstr
13
14 Surg 124, 817-825.
15
16
17

18 Zhou, L., Pomerantseva, I., Bassett, E.K., Bowley, C.M., Zhao, X., Bichara, D.A., Kulig, K.M.,
19
20 Vacanti, J.P., Randolph, M.A., Sundback, C.A., 2011. Engineering ear constructs with a composite
21
22 scaffold to maintain dimensions. Tissue Eng Part A 17, 1573-1581.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure Captions

Figure 1: (a) Schematic representation of a stress-time curve measured on a viscoelastic sample during stress-relaxation indentation. Two strain steps are represented. This curve displays the typical behavior of cartilage during stress-relaxation testing: at each strain step, a sudden stress increase is observed during compression, followed by relaxation to an equilibrium value. Relaxation half-life time $t_{1/2}$ is defined as the time needed for the stress to decrease from its maximum value at the first strain step (σ_i) to $(\sigma_i + \sigma_{eq})/2$, where σ_{eq} is the equilibrium stress at the end of the first strain step. Characteristic relaxation time τ is obtained by fitting an exponential decay function σ_{fitted} to the stress-time curve. $t_{1/2}$ and τ provide measures of fluid flow through the matrix network. (b) Example of a stress-time curve measured on one human auricular cartilage sample (male, 85 year old) during stress-relaxation indentation with 5 successive strain steps (5%, 10%, 15%, 20%, 25%) and a $\varnothing 0.35$ mm indenter.

Figure 2: (a) The bioprinter was placed inside a laminar air flow hood during the biofabrication of a patient-specific ear-shaped BNC implant. The bioprinter consists of (a) a high precision motion system and (b) a micro-dispensing system. (c) The software NI Motion Assistant 2.7 was used to program the controllers of the motion and micro-dispensing systems.

Figure 3: SEM images of BNC with (a) 1% effective cellulose content, (b) 5% and (c) 10%. Cellulose fiber (approximately 30 nm wide) network is visible and fiber spacing is observed to decrease with increasing cellulose concentration.

Figure 4: (a) Instantaneous modulus, E_{in} , of BNC ($n_{total} = 78$) correlates with effective cellulose content ($R = 0.66$), 6.4 ± 3.2 MPa is measured for E_{in} of ear cartilage ($n = 17$). (b) Equilibrium modulus, E_{eq} , of BNC correlates with effective cellulose content ($R = 0.79$), 3.3 ± 1.3 MPa is measured for E_{eq} of ear cartilage. (c) Maximum stress, σ_{max} , of BNC correlates with effective cellulose content ($R = 0.78$), 1.6 ± 0.5 MPa is measured for σ_{max} of ear cartilage. (d) Relaxation half-life time, $t_{1/2}$, of BNC is not influenced by the effective cellulose content, significant differences ($p < 0.05$) are observed between BNC and ear cartilage (0.4 ± 0.1 s and 2.1 ± 1.3 s, respectively). (e) Characteristic relaxation time, τ , of BNC is not influenced by the effective cellulose content, significant differences ($p < 0.05$) are observed between BNC and ear cartilage (3.7 ± 1.0 s and 6.0 ± 2.6 s, respectively).

Figure 5: (a) Transverse slice isolated from a spoiled gradient-echo MRI scan of volunteer's left ear. Auricular cartilage is visible (dashed contour) surrounded by adipose tissue and skin. Plain contour indicates the external outline of the auricle. (b) 3D rendering of the external ear shape (white color) and of the auricular cartilage shape (red color) obtained by manual segmentation of the previous MRI scan. (c) Negative silicone mold used to guide the bacteria during the bacterial culture to reproduce the large-scale features of the outer ear. (d) 3D BNC implant prototype (1% effective cellulose content) produced in the shape of the whole outer ear using the dataset presented in (b).

Figure1

Figure 2
(a)

(b)

(c)

Figure 2

Figure3

Figure4

Figure5

(a)

(b)

(c)

(d)