Journal of Biomechanics 49 (2016) 668–677

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

A puzzle assembly strategy for fabrication of large engineered cartilage tissue constructs

Adam B. Nover [a], Brian K. Jones [b], William T. Yu [a], Daniel S. Donovan [c,d], Jeremy D. Podolnick [c,d], James L. Cook [e], Gerard A. Ateshian [a,b], Clark T. Hung [a,*]

[a] Department of Biomedical Engineering, Columbia University, 351 Engineering Terrace, 1210 Amsterdam Avenue, Mail Code: 8904, New York, NY 10027, USA
[b] Department of Mechanical Engineering, Columbia University, 242 S. W. Mudd, 500 West 120th Street, Mail Code: 4703, New York, NY 10027, USA
[c] Department of Orthopedic Surgery, Mount Sinai West, 1000 Tenth Avenue, New York, NY 10019, USA
[d] Department of Orthopedic Surgery, Mount Sinai St. Luke's, 1111 Amsterdam Avenue, New York, NY 10025, USA
[e] Department of Orthopaedic Surgery, University of Missouri, 1100 Virginia Avenue, DC953.000, Columbia, MO 65212, USA

ARTICLE INFO

Article history:
Accepted 28 January 2016

Keywords:
Articular cartilage
Tissue engineering
Scaling up
Puzzle
Nutrient limitations

ABSTRACT

Engineering of large articular cartilage tissue constructs remains a challenge as tissue growth is limited by nutrient diffusion. Here, a novel strategy is investigated, generating large constructs through the assembly of individually cultured, interlocking, smaller puzzle-shaped subunits. These constructs can be engineered consistently with more desirable mechanical and biochemical properties than larger constructs (~4-fold greater Young's modulus). A failure testing technique was developed to evaluate the physiologic functionality of constructs, which were cultured as individual subunits for 28 days, then assembled and cultured for an additional 21–35 days. Assembled puzzle constructs withstood large deformations (40–50% compressive strain) prior to failure. Their ability to withstand physiologic loads may be enhanced by increases in subunit strength and assembled culture time. A nude mouse model was utilized to show biocompatibility and fusion of assembled puzzle pieces in vivo. Overall, the technique offers a novel, effective approach to scaling up engineered tissues and may be combined with other techniques and/or applied to the engineering of other tissues. Future studies will aim to optimize this system in an effort to engineer and integrate robust subunits to fill large defects.

1. Introduction

Articular cartilage has a limited ability to regenerate, making damage to the tissue especially debilitating (Buckwalter and Mankin, 1998; Sherman et al., 2014). Osteoarthritis (OA), a degenerative disease, affects nearly 27 million Americans, leading to an estimated annual economic cost of $89.1 billion (Bitton, 2009; Lawrence et al., 2008; Leigh et al., 2001). Acute injury to articular cartilage is common and may lead to OA if untreated (Buckwalter and Mankin, 1998; Flanigan et al., 2010), especially when defects become large (Moisio et al., 2009).

Osteochondral allografting is the most common treatment used for large (>2–3 cm^2) articular cartilage defects (Cole et al., 2009; Gortz and Bugbee, 2006; Sherman et al., 2014) in the US. It is a biomimetic technique, used as a long-term solution clinically for over 30 years (Gross et al., 2005, 1975). Allografting shows numerous advantages over alternative techniques. Autologous chondrocyte implantation (ACI) leads to donor site morbidity, requires multiple surgeries, is costly, and is most effective in young, active patients (Lee et al., 2000; Mandelbaum et al., 2007; Sherman et al., 2014; Whittaker et al., 2005). Osteochondral autografting is also associated with donor site morbidity (Lee et al., 2000; Reddy et al., 2007) and consistently successful outcomes are limited to young patients with relatively small (<2 cm^2) defects.

Despite their benefits, allografts are insufficient in supply to meet clinical demand (Mow et al., 1991; Paige and Vacanti, 1995). As an alternative, tissue engineering may provide a cell-based strategy for developing additional grafts for cartilage repair (Johnstone et al., 2013; Lima et al., 2008a). Our laboratories have demonstrated ability to utilize juvenile (Mauck et al., 2000) or adult chondrocytes (Bian et al., 2009a; Kelly et al., 2013; Ng et al., 2010; Nover et al., 2015b) in an agarose hydrogel scaffold system to engineer chondral and osteochondral articular cartilage grafts

* Corresponding author. Tel.: +1 212 854 6542; fax: +1 212 854 8725.
E-mail addresses: abn2106@columbia.edu (A.B. Nover), bkj2106@columbia.edu (B.K. Jones), wty2102@columbia.edu (W.T. Yu), dandonovan@chpnet.org (D.S. Donovan), jpodolnick@chpnet.org (J.D. Podolnick), CookJL@missouri.edu (J.L. Cook), ateshian@columbia.edu (G.A. Ateshian), cth6@columbia.edu (C.T. Hung).

http://dx.doi.org/10.1016/j.jbiomech.2016.01.023
0021-9290/

(Lima et al., 2008a, 2004; Nover et al., 2015a) with native or near-native mechanical and biochemical properties.

Still, in these cases, the engineered grafts were relatively small in size (~⌀4 mm). In order to increase the clinical relevance of engineered grafts, it is critical to scale up their size (Hung et al., 2003). With larger grafts, loads affecting the graft may not be shouldered by surrounding tissue as they are with smaller grafts, making the graft strength even more critical.

A challenge for successful scale up is the inherent diffusion of nutrients and other chemical factors required to support tissue growth. According to Fick's law, diffusion time is proportional to the distance squared (Atkins, 1994). This transport limitation may be exacerbated by nutrient consumption (Buckley et al., 2009) and increased transport resistance arising from dense elaborated tissue within the engineered construct (Leddy et al., 2004). Areas of decreased tissue growth in engineered constructs have been attributed to gradients in various internal nutrient levels including glucose and oxygen (Cigan et al., 2013; Heywood et al., 2006a, 2006b; Nims et al., 2015; Obradovic et al., 1999; Sengers et al., 2005; Zhou et al., 2008). Even in small constructs, this effect has resulted in preferential matrix deposition in peripheral rather than central regions (Kelly et al., 2009, 2006).

Various methods have been investigated for overcoming this diffusion limitation, including dynamic loading (Albro et al., 2008, 2010; Bian et al., 2010; Hung et al., 2004; Lima et al., 2007; Mauck et al., 2000, 2003a, 2003b), perfusion (Davisson et al., 2002), dynamic laminar flow (Vunjak-Novakovic et al., 1999), and orbital motion (Cigan et al., 2014) utilized to increase convection; microbubbles used to create internal reservoirs (Lima et al., 2012); and channels introduced within the tissue to increase surface area and decrease the nutrient path length (Bian et al., 2009a; Buckley et al., 2009; Cigan et al., 2014; Nims et al., 2015).

In the current study, we take a unique approach to this problem by investigating a strategy in which small, interlocking tissue engineered constructs are individually cultured to achieve robust tissue properties, and then assembled to provide a graft capable of covering large defects of variable size and shape in a modular fashion. Proof-of-principle studies are presented that explore a single puzzle piece shape (Fig. 1). Puzzle assemblies are compared to equivalently sized large constructs cultured under the same conditions as puzzle pieces.

2. Methods

2.1. Engineering of puzzle-shaped constructs

Custom punches were designed using Illustrator (Adobe) and SolidWorks (Dassault Systèmes), and then 3D printed (Objet24, Stratasys). An interlocking puzzle-shaped punch was designed to produce a construct with volume similar to that of a ⌀6 mm disk, but with greater surface area. A square-shaped punch was designed to produce single large constructs with equivalent size to an assembly of nine (3×3) puzzle pieces to serve as a control.

Chondrocytes were isolated from articular cartilage harvested from juvenile bovine knees via an 11 hour digestion with 390 U/mL collagenase type IV (Worthington) and slight agitation. Chondrocytes were passaged twice (P2) in Dulbecco's Modified Eagle's Media (DMEM, Invitrogen) containing 10% FBS (Atlanta Biologicals), 1 ng/mL transforming growth factor-beta-1 (TGF-β1, Invitrogen), 5 ng/mL fibroblast growth factor-2 (FGF-2, Invitrogen), and 1% antibiotics/antimycotics (Invitrogen). Chondrocytes were encapsulated in 2% w/v agarose (Type VII, Sigma-Aldrich) at a concentration of 30×10^6 cells/mL, and cast between two glass panes, yielding slabs of 2.3 mm thickness. Constructs were excised from slabs using the 3D printed punches (Groups: “Puzzle” and “Square”).

Constructs were cultured in chemically-defined chondrogenic media: DMEM containing 50 μg/mL L-Proline (Sigma-Aldrich), 100 μg/mL sodium pyruvate (Sigma-Aldrich), 1% ITS+ premix (BD Biosciences), 100 nM dexamethasone (Sigma-Aldrich), 1% antibiotics/antimycotics, and 50 μg/mL ascorbic acid (Sigma-Aldrich). TGF-β3 (R&D Systems) was supplemented for the first 14 days of culture. Per group, medium was scaled by construct volume. Day 0 constructs were evaluated as a baseline. Media was changed 3X/week for 28 days, and then constructs were evaluated (Fig. 2A).

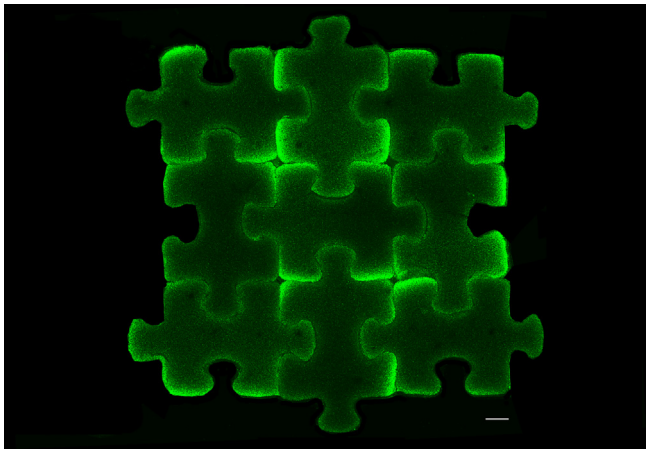

Fig. 1. Schematic of puzzle piece strategy. Cell-seeded agarose stained with calcein for cell visualization. Scale bar: 1 mm.

Local equilibrium Young's modulus (E_Y) was obtained at a central location by curve-fitting the load response of a spherical tip indentation stress-relaxation test (10% strain at 0.5 μm/s, S⌀ 2.31 mm indenter) using the open-source finite element code FEBio (www.febio.org) (Maas et al., 2012). Constructs were modeled as a biphasic material (Mow et al., 1980) with a solid matrix consisting of a neo-Hookean elastic solid (E_Y, and Poisson's ratio, ν), representing the proteoglycan content and a continuous spherical (isotropic) distribution of fibers (fiber modulus ξ and power law exponent β), representing the collagen content (Huang et al., 2012). For reference, spatially-averaged mechanical properties of whole Puzzle constructs were obtained in unconfined compression as previously described (Soltz and Ateshian, 1998), yielding E_Y at 10% unconfined compressive strain and the dynamic modulus (G^*) at 0.01 Hz at 1% strain amplitude superposed over the equilibrated 10% strain configuration. Note that square constructs were not evaluated in this manner due to their large size. In all cases, construct surface area was measured from images using ImageJ (NIH).

For biochemical analysis, constructs were halved along a line of symmetry, and weighed wet (WW), lyophilized, and weighed dry (DW). Constructs were digested in 0.5 mg/mL proteinase-K (MP Biomedicals) for 16 h at 56 °C. A PicoGreen assay (Invitrogen) was used to quantify DNA content (McGowan et al., 2002). A 1,9-dimethylmethylene blue (Sigma-Aldrich) dye-binding assay was used to quantify glycosaminoglycan (GAG) content (Farndale et al., 1982). A colorimetric assay was used to determine orthohydroxyproline (OHP) content (Stegemann and Stalder, 1967). Collagen content was calculated assuming a 1:7.64 OHP-to-collagen mass ratio (Hollander et al., 1994). Biochemical measures were normalized to WW, DW, and DNA content.

Viability was assessed by imaging halved constructs stained with a LIVE/DEAD Assay Kit (Invitrogen) using a confocal microscope (Fluoview FV1000, Olympus).

Halved constructs were fixed for histological analysis using a 5% acetic acid, 3.7% formaldehyde, and 70% ethanol solution for 24 h, and then stored in 70% ethanol. Fixed samples were serially dehydrated in ethanol, embedded in paraffin for visualization of the construct cross-section, sectioned (5 μm), and then mounted on glass slides. Samples were dewaxed, rehydrated, and stained with Fast Green/Safranin O (Sigma-Aldrich) and Picrosirius Red (following 0.5 mg/mL testicular hyaluronidase treatment, Sigma-Aldrich) to visualize GAG and collagen, respectively.

2.2. Assembly of puzzle-shaped grafts

Following 28 days of culture, Puzzle constructs were assembled in a 3×3 arrangement of 9 subunits (Fig. 1). These assemblies and the Square controls were cultured for an additional 21 days (Fig. 2B).

On day 49, Square and assembled Puzzle constructs were evaluated for functionality using compression to failure at a rate of 0.3% of the construct thickness per second (0.3% strain/s at the center point, Fig. 3A) (Tan et al., 2010) using a plano-convex lens (⌀50.8 mm planar end, S⌀65 mm convex surface, Newport Corporation) within a tabletop material testing device (Instron). This novel testing configuration was selected to simulate physiological compression between congruent articular surfaces as the lens would contact nearly the entire construct surface area when its apex penetrated halfway through the construct thickness. Additionally, Puzzle constructs that had been cultured individually for the full 49 days were tested after being assembled fresh prior to testing (Fresh). The energy to failure (ETF) was calculated using a custom MATLAB (Mathworks) program to