Acta Biomaterialia 27 (2015) 286–293

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Porous titanium bases for osteochondral tissue engineering

Adam B. Nover [a], Stephanie L. Lee [a], Maria S. Georgescu [a], Daniel R. Howard [b], Reuben A. Saunders [a], William T. Yu [a], Robert W. Klein [c], Anthony P. Napolitano [c], Gerard A. Ateshian [a,d], 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 Orthopedic Surgery, Mt. Sinai St. Luke's-Roosevelt Hospital, 1000 Tenth Avenue, New York, NY 10019, USA
[c] Stryker Orthopaedics, 325 Corporate Drive, Mahwah, NJ 07430, USA
[d] Department of Mechanical Engineering, Columbia University, 242 S. W. Mudd, 500 West 120th Street, Mail Code: 4703, New York, NY 10027, USA

ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 15 August 2015
Accepted 26 August 2015
Available online 28 August 2015

Keywords:
Tissue engineering
Articular cartilage
Osteochondral grafts
Porous titanium

ABSTRACT

Tissue engineering of osteochondral grafts may offer a cell-based alternative to native allografts, which are in short supply. Previous studies promote the fabrication of grafts consisting of a viable cell-seeded hydrogel integrated atop a porous, bone-like metal. Advantages of the manufacturing process have led to the evaluation of porous titanium as the bone-like base material. Here, porous titanium was shown to support the growth of cartilage to produce native levels of Young's modulus, using a clinically relevant cell source. Mechanical and biochemical properties were similar or higher for the osteochondral constructs compared to chondral-only controls. Further investigation into the mechanical influence of the base on the composite material suggests that underlying pores may decrease interstitial fluid pressurization and applied strains, which may be overcome by alterations to the base structure. Future studies aim to optimize titanium-based tissue engineered osteochondral constructs to best match the structural architecture and strength of native grafts.

Statement of Significance

The studies described in this manuscript follow up on previous studies from our lab pertaining to the fabrication of osteochondral grafts that consist of a bone-like porous metal and a chondrocyte-seeded hydrogel. Here, tissue engineered osteochondral grafts were cultured to native stiffness using adult chondrocytes, a clinically relevant cell source, and a porous titanium base, a material currently used in clinical implants. This porous titanium is manufactured via selective laser melting, offering the advantages of precise control over shape, pore size, and orientation. Additionally, this manuscript describes the mechanical influence of the porous base, which may have applicability to porous bases derived from other materials.

1. Introduction

Focal defects in articular cartilage caused by acute injury have limited healing capacity and may lead to the progression of joint degradation if untreated [1,2]. Such lesions are common: a systematic review found full-thickness focal defects in 36% of athletes [3]. The most common treatment for large focal defects (>2–3 cm^2) is osteochondral (OC) allografting [2,4,5], the only truly biomimetic technique for restoring tissue organization, which has been used clinically for over 30 years [6] to provide a long-term solution [7]. This treatment is preferred over autografts and autologous chondrocyte implantation (ACI), both of which have been associated with donor site morbidity [8–10]. Additionally, ACI requires multiple stages and is more effective in more active, younger patients [4,9–12].

While OC allografts are preferred as a focal defect treatment, their supply is limited [13,14]. Tissue engineering may provide a cell-based alternative repair strategy, generating additional replacement tissues [15]. We have previously reported on the tissue engineering of articular cartilage grafts that achieve native or near-native mechanical and biochemical properties, using juvenile [16] and adult [11,17] chondrocytes in an agarose hydrogel

* Corresponding author.
E-mail addresses: abn2106@columbia.edu (A.B. Nover), sll2164@columbia.edu (S.L. Lee), sinzigeorgescu@gmail.com (M.S. Georgescu), danhoward@chpnet.org (D.R. Howard), reubens@mit.edu (R.A. Saunders), wty2102@columbia.edu (W.T. Yu), Robert.Klein@stryker.com (R.W. Klein), Napolitano@plymouthcma.com (A.P. Napolitano), ateshian@columbia.edu (G.A. Ateshian), cth6@columbia.edu (C.T. Hung).

http://dx.doi.org/10.1016/j.actbio.2015.08.045
1742-7061/

scaffold system. By integrating this technique with a porous bone or bone-like base, we have engineered osteochondral grafts composed of a viable cell-seeded chondral layer atop the base [11,15,18]. Previous research into the bony base has promoted the use of porous metals, such as tantalum, over devitalized bone [15], with successful evaluation of the metal *in vivo* [11].

Recently a selective laser melting technique was reported to fabricate porous titanium structures for orthopaedic applications [19–21]. Titanium is a corrosion resistant, biocompatible material with a high strength-to-weight ratio [21,22]. The selective laser melting technique offers the valuable ability to select pore size and strut orientation to optimize bone ingrowth (100–700 μm pore size, 60–80% porosity, >50 MPa compression strength) [19,23–28] as well as the potential for fabricating anatomically contoured shapes to match native geometry [29]. A similar process is currently utilized by Stryker Orthopaedics (Mahwah, NJ, USA) to manufacture tibial trays and patellar components for clinical use.

The objective of this study was to adopt and evaluate porous titanium dowels, fabricated through selective laser melting, as a bone-like base for tissue engineered OC constructs using an agarose scaffold system [30–32]. Our investigation focused on characterization of the bases' structure, their influence on measured construct mechanical properties, and their compatibility for viable OC tissue growth.

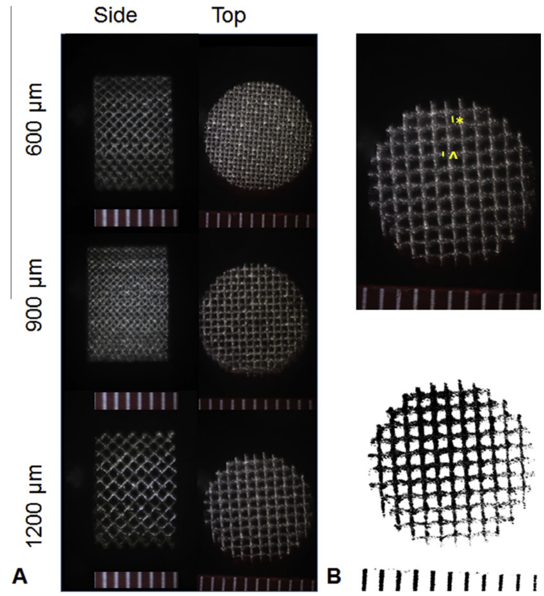

Fig. 1. (A) Representative stereoscopic images of 10 mm porous titanium disks with uniform pore distribution; (B) *Top:* Strut thickness (^) and pore side length (*) shown on a 1200 μm unit cell pore size base; *Bottom:* The same base processed for area fraction measurements. Scale bars in mm.

2. Materials and methods

2.1. Fabrication and structural characterization of porous titanium bases

Cylindrical titanium disks of 4 and 10 mm diameter and 7 mm height were fabricated from commercially pure titanium (Sumitomo, Japan) by Stryker Orthopaedics using an MCP Realizer 2, 250 SLM system (MCP Tooling Technologies, Staffordshire, UK). The system uses an ytterbium fiber laser (600 W power CW, λ = 1.06 μm) with an optical system used to control the movement of the nominal 50 μm diameter focused laser spot on the build area to a positional accuracy of ±5 μm. The system operates in an over pressure argon environment with processing chamber oxygen levels below 0.2%. The atmosphere within the chamber is circulated and filtered to remove process bi-products (titanium nanopowder formed from condensed titanium vapor) from the recycled gas. Parts were built in a layer-wise fashion on a substrate plate connected to an elevator that moves vertically downwards allowing the controlled deposition of powder layers at 50-μm intervals. Upon completion of the build the substrate plate was removed from the build chamber and all un-fused powder was recycled. Test pieces were then cut from the substrate plates. All individual parts were ultrasonically cleaned, dried, and heat treated (1400 °C for 3 h) prior to testing.

Bases were produced with uniform 600, 900, and 1200 μm unit cell pore size with regularly oriented unit cells of struts (0.2 μm thick). Groups are referenced by their unit cell pore size. Representative SEM images have been published by Mullen et al. [19].

As used to characterize trabecular bone, height, diameter, and weight were measured for each construct. Bases were placed in distilled deionized water, degassed, and the submerged weight was measured. From these parameters, the apparent density (ρ) was calculated from the mass and bounding volume. The true density (ρ_s, i.e., density of titanium) was calculated through Archimedes Principle. Metal volume fraction (VF) was calculated as: $VF = \rho/\rho_s$. Porosity (P) was then calculated as $P = 1 - VF = 1 - (\rho/\rho_s)$.

Bases were photographed from the top and side (Fig. 1A) with a stereoscope ($n = 4$). The length of the side of the visible pore square ($n = 6$ pores per base) and strut size ($n = 6$ pores per base) were measured from images using ImageJ (NIH, Bethesda, MD, USA, Fig. 1B). The measured side length was squared to estimate the cross-sectional area of the pore. The images were processed in ImageJ and the pore area fraction was calculated (Fig. 1B).

2.2. Mechanical influence of porous base

Acellular agarose (Type VII, Sigma-Aldrich, St. Louis, MO, USA) disks were cast at 2%, 4%, and 6% w/v of dimensions 4 mm diameter and 2.3 mm thickness. Agarose disks were mechanically tested using a custom device to acquire the equilibrium Young's modulus (E_Y) at 10% unconfined compressive strain of the disks, as previously described [33]. Each construct was tested twice, once on an impermeable surface (chondral) and once centered on top of a 10 mm diameter titanium disk of 600, 900, and 1200 μm pores (fabricated as previously mentioned) with time in between testing procedures for relaxation (osteochondral). The resulting points for each base type were plotted and fit with a linear regression.

The (on top) testing method was validated by comparing the E_Y of acellular chondral samples tested on top of regularly oriented 1200 μm porous titanium base and of osteochondral samples cast into the porous bases ($n = 4–5$) where the gel was integrated into the pores. No significant differences ($p = 0.73$) were observed between the testing configurations, suggesting that the on top test is able to capture the critical features of the gel-pore interactions that influence chondral (region) properties.

The slopes of these regression lines were plotted against their respective unit cell pore size, and the measured pore side length, pore cross-sectional area, pore area fraction, porosity, and apparent density (mean values) for the regularly oriented titanium bases. The appropriate intercept was added when applicable (i.e., a non-porous base would have a porosity of 0). Linear regressions were applied to each plot, and R^2 values were compared.

In order to better understand these results in the context of other types of porous metal bases, this mechanical testing procedure was repeated with randomly oriented 600 μm porous titanium bases [20] and porous tantalum bases (25 mm diameter disk, courtesy Zimmer Biomet, Warsaw, IN, USA) [15], which also