Acta Biomaterialia 63 (2017) 294–305

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Three-dimensional nano-architected scaffolds with tunable stiffness for efficient bone tissue growth

Alessandro Maggi [a,*], Hanqing Li [b,*], Julia R. Greer [a]

[a] Division of Engineering and Applied Science, California Institute of Technology, Pasadena, CA 91125, USA
[b] Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA

ARTICLE INFO

Article history:
Received 16 June 2017
Received in revised form 19 August 2017
Accepted 7 September 2017
Available online 18 September 2017

Keywords:
Two-photon lithography
Scaffold
Micro fabrication
Mechanical characterization
Mineralization
Mechanics

ABSTRACT

The precise mechanisms that lead to orthopedic implant failure are not well understood; it is believed that the micromechanical environment at the bone-implant interface regulates structural stability of an implant. In this work, we seek to understand how the 3D mechanical environment of an implant affects bone formation during early osteointegration. We employed two-photon lithography (TPL) direct laser writing to fabricate 3-dimensional rigid polymer scaffolds with tetrakaidecahedral periodic geometry, herewith referred to as nanolattices, whose strut dimensions were on the same order as osteoblasts' focal adhesions (~2 μm) and pore sizes on the order of cell size, ~10 μm. Some of these nanolattices were subsequently coated with thin conformal layers of Ti or W, and a final outer layer of 18 nm-thick TiO_2 was deposited on all samples to ensure biocompatibility. Nanomechanical experiments on each type of nanolattice revealed the range of stiffnesses of 0.7–100 MPa.

Osteoblast-like cells (SAOS-2) were seeded on each nanolattice, and their mechanosensitve response was explored by tracking mineral secretions and intracellular f-actin and vinculin concentrations after 2, 8 and 12 days of cell culture in mineralization media.

Experiments revealed that the most compliant nanolattices had ~20% more intracellular f-actin and ~40% more Ca and P secreted onto them than the stiffer nanolattices, where such cellular response was virtually indistinguishable.

We constructed a simple phenomenological model that appears to capture the observed relation between scaffold stiffness and f-actin concentration. This model predicts a range of optimal scaffold stiffnesses for maximum f-actin concentration, which appears to be directly correlated with osteoblast-driven mineral deposition.

This work suggests that three-dimensional scaffolds with titania-coated surfaces may provide an optimal microenvironment for cell growth when their stiffness is similar to that of cartilage (~0.5–3 MPa). These findings help provide a greater understanding of osteoblast mechanosensitivity and may have profound implications in developing more effective and safer bone prostheses.

Statement of Significance

Creating prostheses that lead to optimal bone remodeling has been a challenge for more than two decades because of a lack of thorough knowledge of cell behavior in three-dimensional (3D) environments. Literature has shown that 2D substrate stiffness plays a significant role in determining cell behavior, however, limitations in fabrication techniques and difficulties in characterizing cell-scaffold interactions have limited our understanding of how 3D scaffolds' stiffness affects cell response.

The present study shows that scaffold structural stiffness affects osteoblasts cellular response. Specifically this work shows that the cells grown on the most compliant nanolattices with a stiffness of 0.7 MPa expressed ~20% higher concentration of intracellular f-actin and secreted ~40% more Ca and P compared with all other nanolattices. This suggests that bone scaffolds with a stiffness close to that of cartilage may serve as optimal 3D scaffolds for new synthetic bone graft materials.

* Corresponding authors.
E-mail address: maggi@caltech.edu (A. Maggi).

http://dx.doi.org/10.1016/j.actbio.2017.09.007
1742-7061/

1. Introduction

The number of expected osteoporosis-related fractures is predicted to grow by a factor of 7 in the next twenty-five years because of a substantial increase in the ageing population. By 2030, the demand for hip and knee replacements is predicted to increase by 174% and 673%, respectively [1]. This tremendous need for bone prostheses has motivated significant research efforts to develop a more thorough understanding of properties of bone at each level of its hierarchy, with a focus on scaffold-osteoblast interactions at the cellular level [2,3]. Several types of bone grafting scaffolds exist. For example, autografts are bone replacements taken directly from the iliac crest of a patient and transplanted to the target site where they lead to osteointegration, osteoinduction and osteogenesis, which are necessary for a functional bone implant.

Autografts virtually eliminate the risk of implant rejection but they suffer from donor site morbidity and there is limited graft availability. Significant efforts have been directed at developing fully synthetic implants for more than five decades [2]. Commercially available, fully synthetic orthopedic implants are primarily manufactured out of stainless steel and titanium alloys to achieve the required fatigue strength, high strength-to-weight ratio, flexibility, resistance to corrosion, and biocompatibility [3]. The stiffness of these materials is at least two orders of magnitude greater than that of cancellous bone, 0.04–1 GPa [4]. This discrepancy in stiffness between bone and the implant results in insufficient mechanical load transfer from the implant to the surrounding tissues, which leads to a phenomenon known as stress shielding. The bone adapts to these reduced stresses, relative to its natural state, by decreasing its mass, which prevents the bone from anchoring to the implant and leads to implant loosening and eventual failure [4–7]. Hutmacher et al. postulated that an ideal implant should retain durability in the body and have mechanical properties that match those of the natural bone that is being replaced [5]. This remains to be demonstrated experimentally, especially at the cellular level.

To date, research on mammalian cells' ability to exert forces onto a 2-dimensional substrate via stress fibers, which are bundles of polymerized actin, has shown that cells exhibit a bell-shaped sensitivity to changes in substrate stiffness [8,9]. We hypothesize that adhesion and mineralization behavior of bone cells may also exhibit a sensitivity dependence on the stiffness of 3-dimensional 3D) scaffolds [8,10–12]. Identifying an optimal stiffness range for mineralization on 3D scaffolds has the potential to offer quantitative guidelines for the fabrication of bone implants that minimize stress-shielding while maximizing bone growth.

Challenges associated with fabricating complex three-dimensional scaffolds with strut dimensions on the same order as osteoblasts ~10 μm) has rendered existing studies to be limited to a stiffness window ranging from ~10 to 200 kPa [13–16]. Most literature has been focused on studying cell behavior on either 2D substrates or on scaffolds with a narrow range of structural stiffness and strut size of at least one order of magnitude larger than the cell's size which has made the cell-scaffold interaction virtually the same as that on a 2D substrate [5,13,15–19].

3D porous scaffolds with different pore sizes have been shown to offer an excellent platform to mimic natural physiologically relevant microenvironments [18,20,21]. For example, Raimondi et al. fabricated polymeric scaffolds and observed that a minimum pore size of 10 μm was necessary to allow for cell infiltration into their scaffold [18]. Tayalia et al. utilized polymeric scaffolds and showed that cells are more uniformly dispersed inside scaffolds with pore sizes of 52 μm compared to 12 μm [21]. Harley et al. produced col lagen–glycosaminoglycan scaffolds and showed that cell migration and cell speed increased by a factor of 2 when the scaffold's pore size was reduced from 151 to 96 μm [20,22–24]. Most of these studies focused on investigating the relationship between porosity and cellular behavior, with some discussing cell behavior as a function of scaffold stiffness, which likely serves as a key factor in governing osteoblasts' mineralization abilities [25] .

We focus on exploring the dependence of osteoblast-like cells (SAOS-2) on the structural stiffness of porous substrates with a constant pore size. We utilized two-photon lithography, sputtering and atomic layer deposition (ALD) to fabricate periodic, 3-dimensional cellular solids, referred to as nanolattices, with tetrakaidecahedral geometry, measured their structural stiffness, and populated osteoblast-like SAOS-2 cells onto them to study their behavior. The structural modulus of elasticity, or stiffness, E^*, scales with the relative density, $\overline{\rho}$, of a periodic cellular solid, as:

$$E^* = CE_s(\overline{\rho})^m \tag{1}$$

where C is a geometry-dependent proportionality constant, E_s is the elastic modulus of the solid that comprises the solid [26,27] and m is a topology-dependent power law coefficient. The relative density is defined as the volume fraction of the solid material, V_s, divided by the representative volume of the unit cell, V_{uc} [28,29]:

$$\overline{\rho} = \frac{V_s}{V_{UC}} \tag{2}$$

Relative density is a function of unit cell topology, mean pore size, U, and the ratio of beam-length to beam-radius, L/R, as shown in Fig. 1(i). The relative density of the nanolattices in this work, calculated using Solidworks software (Dassault Systems), ranged from 0.14% to 12.2%.

The pore size, U, was maintained constant at 25 μm for all nanolattices in this work to isolate the effects of the scaffolds' structural stiffness, which was varied by depositing different material coatings onto the original polymer nanolattices (Fig. 1). We were able to achieve a range of structural stiffnesses that spans over two orders of magnitude, from ~0.7 MPa to 100 MPa, which covers a region that had not been previously explored: existing literature on scaffolds with similar sizes explored the stiffness range of ~10–200 kPa.

SAOS-2 cells were seeded on the nanolattices, and the cells' f-actin concentration was measured after a 48-h growth period in mineralization media. Longer periods of growth, up to 12 days, were conducted to characterize the relationship between scaffold stiffness and cells' mineralization ability.

2. Materials and methods

2.1. Sample preparation

All scaffolds were fabricated via TPL direct laser writing (DWL), which employs a femtosecond-pulsed laser that is rastered in space to selectively cross-link a negative tone photoresist, IP-Dip (Nanoscribe GmbH), into a designed structure. The resulting polymer nanolattices were subsequently coated with different materials to create scaffolds that are comprised of 4 different material systems shown in Fig. 1(i).

Material system (A) was fabricated by first coating the polymer scaffold with an 18 nm-thick layer of TiO_2 deposited via ALD and then slicing off the sample edges along each face using a focused ion beam (FIB) (FEI Nova 200 Nanolab) at 30 KeV and 5 nA. The samples were then placed into an O_2 plasma etcher at 0.6 mbarr and 100 W (Diener GmbH) for 24 h to etch away the original scaffold and to produce a hollow TiO2 nanolattice (Fig. 1(ii) and (iv)).