Materials Science and Engineering C 77 (2017) 219–228

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

The effect of pore size and porosity on mechanical properties and biological response of porous titanium scaffolds

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ARTICLE INFO

Article history:
Received 22 December 2016
Received in revised form 23 March 2017
Accepted 26 March 2017
Available online 27 March 2017

Keywords:
Porous Ti
Space-holder
Young's modulus
Compressive strength
Cell behaviour
Cell attachment
Cell proliferation
Cortical bone
Trabecular bone

ABSTRACT

The effect of pore size and porosity on elastic modulus, strength, cell attachment and cell proliferation was studied for Ti porous scaffolds manufactured via powder metallurgy and sintering. Porous scaffolds were prepared in two ranges of porosities so that their mechanical properties could mimic those of cortical and trabecular bone respectively. Space-holder engineered pore size distributions were carefully determined to study the impact that small changes in pore size may have on mechanical and biological behaviour. The Young's moduli and compressive strengths were correlated with the relative porosity. Linear, power and exponential regressions were studied to confirm the predictability in the characterisation of the manufactured scaffolds and therefore establish them as a design tool for customisation of devices to suit patients' needs. The correlations were stronger for the linear and the power law regressions and poor for the exponential regressions. The optimal pore microarchitecture (i.e. pore size and porosity) for scaffolds to be used in bone grafting for cortical bone was set to <212 μm with volumetric porosity values of 27–37%, and for trabecular tissues to 300–500 μm with volumetric porosity values of 54–58%. The pore size range 212–300 μm with volumetric porosity values of 38–56% was reported as the least favourable to cell proliferation in the longitudinal study of 12 days of incubation.

1. Introduction

An optimum balance between mechanical properties and microstructure (i.e. porosity and pore size) must be achieved to ensure successful long-term implantation of load-bearing orthopaedic devices. Replicating the mechanical properties of bone is crucial to avoid: (i) 'stress shielding' that weakens the bone tissue near the implantation region, and (ii) a loosening effect from the lack of cell tissue integration derived from non-porous interfaces [1]. The mechanical properties of human bone tissue depend strongly on anatomical location and bone tissue type (e.g. cortical or trabecular). Elastic modulus and compressive strength for cortical bone have been reported in the ranges of 7–20 GPa, more typically 15–19 GPa, and 100–250 MPa, more typically 180–210 MPa, respectively [2–4]. The values for trabecular bone are 1.5–11.2 GPa, more typically 2–5 GPa, and 11–24 MPa, respectively [5–8]. Bone ingrowth requires that the bone graft microstructure is osteoconductive (i.e. it guides the bone ingrowth by providing the cells with a structure/scaffold that promotes cell adhesion and proliferation) and leads to osseointegration of the implant (i.e. the sequential cell differentiation and maturation to create cells within the scaffold) [9]. A vast body of literature has been published reporting optimum pore size range to support growth of cells in regenerative applications. For load-bearing bone grafting applications the pore size range has been established at 50–500 μm [10]. Some authors report that pores larger than 300 μm will promote vascularisation [11–13], with cells spanning directly across pores smaller than 150 μm and occupying pores larger than 200 μm [14]. An optimum size cannot be concluded from the results as this value seems highly dependent on the conditions of the study (e.g. 25 and 200 μm had the most positive effect in a range 25–500 μm [15], 325 μm when studying 85–325 μm [16], 400 μm was preferred when the range studied was 75–900 μm [17] and 600 μm in a 300–1000 μm range [12]).

Titanium is broadly used as a material for orthopaedic devices due to its good corrosion resistance and biocompatibility when implanted. Matching mechanical properties of the Ti implant to those of bone involves lowering the stiffness of the material almost an order of magnitude (i.e. from ~110 GPa to ~20 GPa [18]). The stiffness of solid Ti can be lowered by introducing a porous structure which is also favourable for osteoconductivity and osseointegration. Techniques such as foaming, replica, rapid prototyping or sintering with space holders have been reported in literature [18–20]. The latter presents advantages that makes it a preferred method for the fabrication of controlled porosity scaffolds. These are easiness in handling Ti raw material, which is

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http://dx.doi.org/10.1016/j.msec.2017.03.249
0928-4931/

highly oxygen-reactive, lower-than-melting temperatures employed in its processing and a fine control on volumetric porosity that resembles that of natural structures such as bone, preferred in bioengineering substrates and without straight edges [21]. Shape holder materials such as ammonium hydrogen carbonate, urea, sodium fluoride and chloride, saccharose and PMMA have been used in the manufacture of porous materials to control porosity and pore size [20,22–24]. Therefore the strength-to-weight ratio can be optimised to match the mechanical properties of bone and these cavities engineered to promote cell proliferation, which results in anchoring of the bone graft in place to minimise loosening in the mid- and long-term.

Once the mechanical properties of host tissue-implanted substrate have been matched, it has been demonstrated that subtle changes in pore size may have significant effects on cell adhesion and proliferation [16]. In this study the optimal pore microarchitecture (i.e. pore size and porosity) for scaffolds to be used in bone grafting for cortical and trabecular tissues is investigated. Porous scaffolds were manufactured in two ranges of porosities so that their mechanical properties could mimic those of cortical and trabecular bone. The mechanical properties (i.e. Young's modulus and compressive strength) were correlated to the relative porosity and regressions then established in a novel attempt to characterise the pore size distribution with existing porous models. Pore size ranges were engineered and studied for how they affect initial cell attachment and subsequent cell proliferation in a longitudinal study to 12 days.

2. Materials and methods

2.1. Materials

Commercially available elemental Ti powder (Alfa Aesar, MA, 99.5% purity, ≤45 μm, −325 mesh) was used as the main matrix constituent and Ammonium carbonate (NH_4HCO_3) (Fisher, 99% purity) was the space holder.

2.2. Porous scaffolds preparation

Ammonium carbonate particles were sieved (Retsch sieve shaker AS 400 control, Germany) to four particle ranges as follows: 45–106 μm (referred as 'range 1' thereafter), 106–212 μm (range 2), 212–300 μm (range 3) and 300–500 μm (range 4). The Ti powder was mixed with the space holder. Given the particle size range of the ammonium carbonate, the powder to space holder volume ratio used was adjusted in order to contain two porosity ratios (nominally 55% and 70%). In this way porosity and pore size could be controlled independently. Ti specimens without space holder were also fabricated as control (i.e. non-porous, sintered samples). The green bodies were fabricated by uniaxially cold compacting (Atlas Autotouch Press 40, Specac UK) the Ti/space holder powder mixture at a pressure of 250 MPa into cylindrical green compacts of diameter 14 mm and height 8 mm. These were subjected to a calcination process at 100 °C dwelling for 10 h to sublimate the space holders that left voids behind. The sintering process followed in a furnace (Lenton Thermal Designs, UK) equipped with an EcoCube diaphragm pump (Pfeiffer, UK) that achieved a high vacuum (i.e. $<2.10^{-5}$ mbar). The specimens were heated at a rate of 5 °C/min to 1200 °C, allowed to dwell for 12 h and then cooled down to room temperature at the same rate. The samples were wet ground and polished using an incremental regime from 240- to 1200-grit silicon carbide cloth at 10 min interval each and finally air dried.

2.3. Characterisation of the sintered porous scaffolds

The density of the porous scaffolds (ρ^*), relative density (ρ^*/ρ_s), total porosity (P, %vol) and open porosity (P_o) were calculated from the mass-to-volume ratio, the ratio between the density of the porous scaffold versus the density of the sintered, non-porous scaffold, the ($1-\rho^*/\rho_s$) relationship and the pore volume to total volume ratio, respectively. Pore volume was measured using the Archimedes' method in which the volume displaced by the scaffold corresponded to the matrix volume, and therefore, the closed porosity. Slices of the samples (1.5 mm thickness, Buehler Low-Speed saw with oil as a lubricant and without further polishing) were photographed (Nikon D4, 1.6 s exposure time, ISO-100, 60 mm focal length, f3.8 aperture) and subjected to image analysis (ImageJ, NIH, USA). Pore dimensions were measured from the micrographs obtained using a Nikon Optiphot microscope (Nikon, Japan) with a GXCAM 5 camera (GXOptical, UK), using GXCapture software (GTVision, UK). A constituents and contamination analysis was performed using an Energy Dispersive X-ray Spectrometer (SEM, Hitachi TM3030, Japan/Oxford Instruments Swift ED3000 Silicon drift detector (SDD), UK) which analysed the surface of the scaffolds. Mechanical properties of the scaffolds were obtained under compressive conditions at room temperature using a 3369 Instron bench top universal testing machine (Instron, UK). Samples were loaded at a constant speed of 1 mm/min. The compressive elastic modulus (i.e. Young's modulus) was obtained from the gradient of the elastic region and the compressive strength from the 'yield point' at which densification or microfracture commenced.

2.4. In vitro studies

2.4.1. Preparation of the scaffolds and cell culture

Slices of the scaffolds of nominal porosity 55% (A) and 70% (B) in the pore ranges 1–4 (i.e. A1, A2, A3, A4 and B1, B2, B3, B4) were cleaned, informed by the cleaning protocol reported in [25], to remove unwanted oil impurities and the outmost oxide layer. Specimens were first stirred in soapy hot water (2 h), soaked in bleach (2 h) and then oven treated (200 °C for 1 h on each side) to remove contamination of a carbon nature. They were then ultrasonically cleaned while immersed in acetone for 1.5 h and finally stored in 2-isopropanol at 4 °C for until further use. Prior to biological tests the samples were sterilized by autoclaving at 121 °C for 1 h and thoroughly rinsed in deionised sterile water.

Culture media was prepared using MEM enriched with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% non-essential amminoacids (Sigma, UK). Human bone osteosarcoma cell line 143B (ECACC no. 91112502) were defrosted and seeded in standard flasks. They were incubated in a 5% CO_2 atmosphere at 37 °C in an incubator (Thermo Scientific Heracell™ 150, UK). Media was changed every 3 days for the entire duration of the experiment.

2.4.2. Cellular viability and proliferation

Specimens A1–4 and B1–4 were placed in 24 low-adherence multiwell plates (Corning Costar®, UK) and soaked in 1.5 mL of the culture media for 2 h until cell seeding. Non-porous slices were used as control blanks. Cells (5000 cells per well) were seeded onto each of the specimens and the control wells. Cells were let to attach for 2 h and culture wells were refilled with 2 mL of enriched medium. Cell viability and morphology were tested at 3, 7, 12 days of incubation.

2.4.3. Presto Blue assay for cell viability

The Presto blue viability assay contains a cell permeable resazurin-based solution that functions as a cell viability indicator by using the reducing power of living cells to quantitatively measure their proliferation. This analogue allowed the quantification of initial attachment,

Table 1
Chemical composition (EDS) results.

Element	Ti	C	Al	Si	Br
%wt (SD)	96.050 (1.831)	4.265 (0.679)	2.064 (0.325)	1.078 (0.003)	1.232 (0.011)