



# The effect of pore geometry on the in vitro biological behavior of human periosteum-derived cells seeded on selective laser-melted Ti6Al4V bone scaffolds

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## ARTICLE INFO

### Article history:

Received 13 December 2011

Received in revised form 28 March 2012

Accepted 2 April 2012

Available online 7 April 2012

### Keywords:

Selective laser melting

Ti6Al4V

Geometry

In vitro

Bone tissue engineering

## ABSTRACT

The specific aim of this study was to gain insight into the influence of scaffold pore size, pore shape and permeability on the in vitro proliferation and differentiation of three-dimensional (3-D) human periosteum-derived cell (hPDC) cultures. Selective laser melting (SLM) was used to produce six distinct designed geometries of Ti6Al4V scaffolds in three different pore shapes (triangular, hexagonal and rectangular) and two different pore sizes (500  $\mu\text{m}$  and 1000  $\mu\text{m}$ ). All scaffolds were characterized by means of two-dimensional optical microscopy, 3-D microfocus X-ray computed tomography (micro-CT) image analysis, mechanical compression testing and computational fluid dynamical analysis. The results showed that SLM was capable of producing Ti6Al4V scaffolds with a broad range of morphological and mechanical properties. The in vitro study showed that scaffolds with a lower permeability gave rise to a significantly higher number of cells attached to the scaffolds after seeding. Qualitative analysis by means of live/dead staining and scanning electron microscopy showed a circular cell growth pattern which was independent of the pore size and shape. This resulted in pore occlusion which was found to be the highest on scaffolds with 500  $\mu\text{m}$  hexagonal pores. Interestingly, pore size but not pore shape was found to significantly influence the growth of hPDC on the scaffolds, whereas the differentiation of hPDC was dependent on both pore shape and pore size. The results showed that, for SLM-produced Ti6Al4V scaffolds with specific morphological and mechanical properties, a functional graded scaffold will contribute to enhanced cell seeding and at the same time can maintain nutrient transport throughout the whole scaffold during in vitro culturing by avoiding pore occlusion.

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## 1. Introduction

For the treatment of large or non-healing bone defects, no optimal solution exists to date. All currently available therapies have important drawbacks. To resolve this clinical problem, there is a strong tendency towards regeneration of the bone tissue in the defect. This new approach, called tissue engineering (TE), combines the advantages of autografts and allografts by using cell-seeded porous structures (bone scaffolds), and eliminates problems such as donor site scarcity, immune rejection and pathogen transfer [1]. It is generally accepted that bone scaffolds should have at

least the following characteristics [2,3]: (i) biocompatibility; (ii) mechanical properties that match those of the host tissue; and (iii) a highly porous and interconnected structure to allow cell migration, proliferation and differentiation and nutrient–waste exchange. It is also clear that all these parameters will define the quality of the bone scaffold. However, the optimal porous structure is still not defined. It is therefore necessary to investigate the effects of these scaffold properties on biological outcome. For this, the production of scaffolds with high controllability and repeatability in terms of mechanical and morphological parameters is strongly demanded. Conventional scaffold fabrication techniques such as fiber bonding, gas foaming and freeze drying offer limited flexibility in controlling the morphology of the scaffold [4–6]. In contrast, additive manufacturing (AM) techniques are capable of producing porous structures with a controlled architecture, owing to their layer-wise building and their direct link with a computer-aided design (CAD) model [7–11]. In fact, AM techniques have been

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