

## Biocompatibility and osteogenic potential of human fetal femur-derived cells on surface selective laser sintered scaffolds

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### Abstract

For optimal bone regeneration, scaffolds need to fit anatomically into the requisite bone defects and, ideally, augment cell growth and differentiation. In this study we evaluated novel computationally designed surface selective laser sintering (SSLS) scaffolds for their biocompatibility as templates, *in vitro* and *in vivo*, for human fetal femur-derived cell viability, growth and osteogenesis. Fetal femur-derived cells were successfully cultured on SSLS-poly(D,L)-lactic acid (SSLS-PLA) scaffolds expressing alkaline phosphatase activity after 7 days. Cell proliferation, ingrowth, Alcian blue/Sirius red and type I collagen positive staining of matrix deposition were observed for fetal femur-derived cells cultured on SSLS-PLA scaffolds *in vitro* and *in vivo*. SSLS-PLA scaffolds and SSLS-PLA scaffolds seeded with fetal femur-derived cells implanted into a murine critical-sized femur segmental defect model aided the regeneration of the bone defect. SSLS techniques allow fabrication of biocompatible/biodegradable scaffolds, computationally designed to fit any defect, providing a template for cell osteogenesis *in vitro* and *in vivo*.

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### 1. Introduction

The majority of bone fracture defects repair spontaneously with minimal treatment or scarring [1]. However, in clinical settings where the defect is too large for natural repair, such as bone defects and non-unions caused by trauma, tumors, osteomyelitis as well as maxillofacial bone loss, a replacement material is needed to help bridge the gap

and enhance the osteogenic healing process. Thus, there is a clinical need for anatomically shaped biomaterials to repair voids of bone loss in bone defects [1]. Recent tissue engineering advances have led to the possibility of successful repair and restoration of function in damaged or diseased skeletal tissues [2,3]. A variety of porous scaffold polymers (naturally derived or synthetic matrices) [4] have shown significant potential for bone tissue engineering applications. However, to be truly effective, these scaffolds must be biologically compatible to reduce adverse inflammatory reactions, augment cellular ingrowth and, ideally, fit anatomically within the bone defect. Thus, fabrication of bone

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replacement materials and scaffolds for tissue engineering applications would benefit significantly from manufacturing techniques that control, precisely, the scaffold architecture both internally and externally.

Current implant procedures typically require bone replacement materials to be cut, shaped and formed prior to implantation, resulting in an expensive and time-consuming process. Furthermore, conventional processing techniques are limited in the geometric structures available, leading to a lack of control on the development of the internal architecture such as pore size, porosity and permeability. These various limitations can compromise the mechanical, biological and stability properties of the engineered implant [5]. To date, scaffold-forming techniques have centered around processing strategies rather than engineering design, and thus, typically, lack precision and reproducibility.

Solid freeform fabrication (SFF) techniques have a number of distinct advantages over conventional construction of scaffolds [6–8], including the ability to build objects in an additive, layer-by-layer method, directly from computer-assisted design using three-dimensional (3-D) digital data of a representative part [9]. One such SFF technique which has had some success in creating bone tissue-engineered constructs is selective laser sintering (SLS) [10–13]. SLS is an increasingly important method for creating complex-shaped prototypes that allows for the design of anatomically shaped scaffolds with defined pore size, porosity, topography and strength. SLS reproduces scaffold constructs from 3-D digital data including magnetic resonance imaging or X-ray tomography via a computer-controlled scanning laser beam which sequentially fuses regions in a powder bed layer-by-layer. However, conventional SLS polymer powders are exposed to temperatures which are prohibitively high for many biodegradable materials and bioactive species [7,14,15], limiting their biomedical application.

Popov et al. [16] have developed a new method for bioactive and bioresorbable scaffold fabrication based on a modified and enhanced SLS procedure—surface selective laser sintering (SSLS). SSLS is initiated by melting only the polymer particle surface but not poly(D,L-lactic) acid (PLA) powder, which does not absorb ( $\sigma_{ab} < 0.1 \text{ cm}^{-2}$ ) near-infrared ( $\lambda = 0.97 \text{ }\mu\text{m}$ ) laser radiation. The laser light is absorbed by a small quantity of homogeneously distributed biocompatible carbon black (CB) microparticles [17] added to the PLA on the surface of each particle, leading to localized surface heating. Altering the laser intensity and laser beam scanning speed has enabled reproducible fabrication of 3-D polymer scaffolds with specific shape and internal structure. In addition, the bulk of each polymeric particle is not melted, thus this surface sintering has the potential to enable the inclusion of bioactive species within the polymer particles [18].

This technology offers new approaches to develop novel scaffold implants to engineer bone tissue. The objective of these studies was to examine the suitability and biocompatibility of these prototype SSLS-PLA composite scaffolds

for promoting human fetal femur-derived cell adhesion, growth, viability, and osteogenesis in vitro and in vivo.

## 2. Materials and methods

### 2.1. Materials

Fetal calf serum (FCS) was purchased from Invitrogen (Paisley, UK). Vybrant<sup>®</sup> carboxy fluorescein diacetate, succinimidyl ester (CFDA SE) cell tracer kit, Cell Tracker Green<sup>™</sup> (CMFDA) and ethidium homodimer-1 were purchased from Molecular Probes (Leiden, The Netherlands). PLA (Medisorb<sup>®</sup> 100 DL HI IV,  $M_w = 108 \text{ kDa}$ , polydispersity = 1.4) was purchased from Alkermes (Boston, MA, USA).  $\alpha$ -Minimum essential medium ( $\alpha$ -MEM), dexamethasone, ascorbate and alkaline phosphatase kits and all other tissue culture reagents were purchased from Sigma–Aldrich (Poole, UK) unless stated.

### 2.2. Fabrication of SSLS-PLA scaffolds

PLA was ground to a fine powder (mean particle diameter  $\sim 200 \text{ }\mu\text{m}$ ) at dry-ice temperatures using a pestle and mortar before mixing with a small amount ( $< 0.1 \text{ wt.}\%$ ) of CB microparticles (mean size  $\sim 360 \text{ nm}$ , surface area  $\sim 100 \text{ m}^2 \text{ g}^{-1}$ ). Using an experimental prototype SLS-80 laser, PLA scaffolds were fabricated with the desired architecture designed and produced by ILIT RAS (Troitsk/Shatura, Moscow Region, Russia). This prototype is based on a continuous wave (CW) fiber diode laser LS-097 (IRE-Polus Ltd., Moscow, Russia) emitting at  $\lambda = 0.97 \text{ }\mu\text{m}$  with a maximum power of 10 W. This radiation was delivered to the PLA/CB powder bed using a beam delivery system including a quartz fiber, magnifying objective, focusing lens and X–Y computer-controlled scanner, resulting in a  $\sim 200 \text{ }\mu\text{m}$  diameter laser spot on the polymer particles. Only CB particles absorb radiation at this wavelength, allowing specific surface melting and powder fusion, and thus creating a scaffold (size =  $5 \text{ mm}^2$ ; layer thickness =  $300 \text{ }\mu\text{m}$ ;  $\sim 13$  layers). The threshold laser intensity for reliable sintering of the polymer particles with CB at the scan speed  $3 \text{ mm s}^{-1}$  was approximately  $I_t \approx 100 \pm 20 \text{ W cm}^{-2}$ .

### 2.3. Isolation and culture of human fetal femur-derived cells

Human fetal femurs were obtained and isolated following termination of pregnancy according to guidelines issued by the Polkinghome Report and with ethical approval from the Southampton & South West Hampshire Local Research Ethics Committee (LREC No. 296100). Primary cultures of human fetal femur-derived cells were prepared as previously described and characterized [19]. Briefly, fetal age was determined by measuring fetal foot length (range of 7.5–11 weeks post-conception) and the femurs were placed in sterile  $1 \times$  phosphate-buffered saline ( $1 \times$  PBS) prior to removal of surrounding skeletal muscle. Femurs were dissected and cell isolation was achieved by a

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