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The effect of anisotropic architecture on cell and tissue infiltration into tissue engineering scaffolds

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Abstract

A common phenomenon in tissue engineering is rapid tissue formation on the outer edge of the scaffold which restricts cell penetration and nutrient exchange to the scaffold centre, resulting in a necrotic core. To address this problem, we generated scaffolds with both random and anisotropic open porous architectures to enhance cell and subsequent tissue infiltration throughout the scaffold for applications in bone and cartilage engineering. Hydroxyapatite (HA) and poly(D,L-lactic acid) ($P_{DL}LA$) scaffolds with random open porosity were manufactured, using modified slip-casting and by supercritical fluid processing respectively, and subsequently characterised. An array of porous aligned channels (400 μm) was incorporated into both scaffold types and cell (human osteoblast sarcoma, for HA scaffolds; ovine meniscal fibrochondrocytes, for $P_{DL}LA$ scaffolds) and tissue infiltration into these modified scaffolds was assessed in vitro (cell penetration) and in vivo (tissue infiltration; HA scaffolds only). Scaffolds were shown to have an extensive random, open porous structure with an average porosity of 85%. Enhanced cell and tissue penetration was observed both in vitro and in vivo demonstrating that scaffold design alone can influence cell and tissue infiltration into the centre of tissue engineering scaffolds.

Keywords: Hydroxyapatite; Poly(lactic acid); Aligned macroarchitecture; Bone tissue engineering; Cartilage tissue engineering; Scaffold

1. Introduction

The clinical need to develop improved material structures for reconstructive surgery and tissue engineering has been the driving force behind both academic and commercial research into regenerative medicine [1–3]. Many tissue engineering strategies involve the fabrication of a material scaffold that plays an important role in supporting initial cell attachment and subsequently in the guidance of tissue formation whilst providing mechanical stability. These devices can be produced from synthetic and naturally derived polymers, as well as inorganic materials, and are widely used in the field of tissue engineering [4–6].

Much research within tissue engineering focuses on the design of the scaffold to ensure appropriate tissue growth is encouraged [7]. However, a common problem encountered when using such scaffolds for tissue engineering is the rapid formation of tissue on the outer edge, which leads to the development of a necrotic core due to the limitations of cell penetration and nutrient exchange [8,9]. Most tissues possess a network of blood and capillary vessels that perform this function in vivo but engineering such a complex construct in vitro is challenging and as yet, has eluded tissue engineers [10,11]. Even those tissues that are avascular, such as cartilage, can prove challenging to engineer if the in vivo nutrient supply cannot be adequately simulated in vitro [8].

To date many different strategies have been employed to overcome this issue. A common approach is to utilise sophisticated culture systems to perfuse culture media

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around and/or through the scaffold [12–14]. Although these bioreactors have been successful and are increasingly vital to tissue engineering in vitro, this field is still developing appropriate systems for good tissue growth. However, even in an optimised in vitro culture system, there is still a need to ensure tissue growth occurs evenly throughout the construct. Furthermore, for strategies where the scaffold alone will be implanted and the body used as the bioreactor, it is important to ensure that native tissue can infiltrate the whole scaffold to ensure adequate integration of the construct [15]. Another strategy to encourage tissue formation and cell differentiation within scaffolds is to incorporate growth factors which can also act as chemotactic agents to encourage cell migration [3]. Enhanced tissue formation using this method has been demonstrated with the $P_{DL}LA$ scaffolds presented in this study [16] but there is still scope to improve this further by potentially using a dual strategy.

An alternative method of addressing these issues is to incorporate a design within the scaffold that will improve nutrient and cell transfer to the scaffold centre, both in vitro and in vivo. This is especially important whilst the challenge of incorporating a vasculature into tissue engineered constructs (that require a blood supply) prior to implantation is being resolved to ensure necrosis does not occur within the scaffold during the establishment of a native vasculature. Recent studies have reported the incorporation of aligned channels into the general structure of the scaffold in order to achieve this goal. This has been demonstrated using fabrication methods such as phase-separation techniques [17], solid free-form fabrication [18] and rapid prototyping [19]. The resulting scaffolds with aligned channels essentially provided a thoroughfare for both cell and nutrient flow. In all these studies, where either in vitro and/or in vivo studies were carried out, cell/tissue penetration into the scaffold was observed. However, it is worth noting that some of these studies still reported greater cell/tissue infiltration at the periphery of the scaffold rather than in the centre and a comparison to nonarchitectured scaffolds was not made [19–21].

Previous studies from our laboratories have demonstrated that the optimum channel diameter for human osteosarcoma cell penetration into porous hydroxyapatite (HA) scaffolds was approximately 400 μm [22]. This work is taken further in this paper where the strategy of incorporating anisotropic macroarchitecture within porous scaffolds is assessed both in vitro and in vivo in two scaffold types suitable for bone and cartilage tissue engineering.

2. Materials and methods

2.1. Scaffold fabrication

2.1.1. Ceramic hydroxyapatite scaffolds

HA scaffolds were manufactured using a modified slip-casting method as described previously [22,23]. For scaffolds with aligned macroarchitecture, stainless steel needles (600 μm diameter) were inserted, at the green state, perpendicular to the cylindrical face (1 centrally located channel for in vitro studies and 13 channels for mechanical testing and in vivo studies). After sintering in air at 1350 °C for 3 h, the ceramics for biological studies were washed by ultrasonic agitation in ultra pure water for 15 min, sterilised by autoclaving at 120 °C for 20 min, and stored at room temperature until required.

2.1.2. $P_{DL}LA$ scaffolds

$P_{DL}LA$ scaffolds were fabricated using supercritical CO_2 ($scCO_2$), as previously described [24]. To introduce channels into the random, porous scaffold structure, a computer controlled drill system (developed in-house) was used to drill 21 channels of 400 μm diameter through each scaffold. Scaffolds were stored at room temperature until required. Sterilisation for cell studies involved immersion of the scaffold in 5% (v/v) AB/AM in PBS pH 7.4 for 2 h, washing overnight at 4 °C in sterile dH_2O with gentle agitation and UV irradiation for 15 min on each side. Scaffolds were collagen coated with 10 μg/mL type I collagen (Sigma, UK) in PBS pH 7.4 overnight at 4 °C prior to seeding to encourage cell attachment.

2.2. Scaffold characterisation

2.2.1. Scaffold morphology in 3D

The scaffolds were imaged using a high-resolution micro-computed tomography system (micro-CT 40, Scanco Medical, Switzerland). The scanner was set to a voltage of 55 kVp and a current of 145 mA. Samples were scanned at 8 μm voxel (3D pixel) resolution with an integration time of 300 ms to produce 3D raw data files. For scaffold imaging, the raw 3D tomographic images for all samples were evaluated using the same threshold value (60) to remove relatively low grey-scale or background values. Scaffold porosity, mean pore and window diameters were calculated using the Scanco software (associated with the micro-CT).

2.2.2. Porosity determination

The total porosity of both scaffold types (10 mm diameter × 5 mm height) was determined using micro-CT (as described above). Closed porosity was determined using a Micromeritics AccuPyc 1330 Gas Pycnometer (Micromeritics, UK). Scaffolds and unprocessed polymer/ceramic were dried overnight at 37 °C prior to absolute density measurements from which closed porosity was calculated [25].

2.2.3. Pore and window morphology

To observe pore and window morphology, scaffolds were sputter coated with gold for 8 min for HA scaffolds or 4 min for $P_{DL}LA$ scaffolds under an argon atmosphere in a Blazers SCD 030 sputter coater unit and visualised using scanning electron microscopy (SEM) with a Philips XL30 scanning electron microscope (10 kV). Channel diameter measurements were made using the Philips image analysis software associated with the electron microscope. The average pore and window size for both scaffold types were calculated by micro-CT (as described above) using the methods described in Moore et al. [26].

2.2.4. Mechanical testing

Mechanical strength data were obtained through compression testing using a Lloyd tensile testing machine (Model M30K) at a cross head speed of 1 mm/min and an extension of 5 mm. HA (four samples) and $P_{DL}LA$ (10 samples) scaffolds with and without aligned channels were tested; those with channels were tested in the direction of the channel. Variation in scaffold diameter, thickness and weight were noted.

2.3. Cell isolation and culture

2.3.1. Ovine meniscal fibrochondrocytes

Ovine meniscal fibrochondrocytes (OMC) were isolated from young ovine stifle joints (supplied by Broomhall's butchers, Gloucester, UK) according to the method described by Collier and Ghosh [27]. Isolated