

Cell viability, proliferation and extracellular matrix production of human annulus fibrosus cells cultured within PDLLA/Bioglass[®] composite foam scaffolds *in vitro*

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Abstract

The objective of this study was to assess cell viability, attachment, morphology, proliferation, and collagen and sulphated glycosaminoglycan (s-GAG) production by human annulus fibrosus (HAF) cells cultured *in vitro* in poly(D,L-lactide) (PDLLA)/Bioglass[®] composite foams. PDLLA foams with different percentages (0, 5 and 30 wt.%) of Bioglass[®] particles were prepared by thermally induced phase separation (TIPS) and characterized by scanning electron microscopy (SEM). HAF cell viability in the PDLLA/Bioglass[®] foam was analysed using Live/Dead staining. HAF cell attachment was observed using SEM. An assessment of cell proliferation was conducted using the WST-1 assay. The level of s-GAG and collagen produced by HAF cells was quantified using the 1,9-dimethylmethylene blue (DMMB) assay and Sircol[™] assay after 4 weeks of culture. The presence of collagen types I and II within the PDLLA/Bioglass[®] composite foams was analysed using immunohistochemistry. Live/dead staining showed that many viable HAF cells were present on the top surface of the foams as well as penetrating into the internal pore structure, suggesting that the PDLLA/Bioglass[®] composite materials are non-toxic and that the presence of Bioglass[®] particles within PDLLA scaffolds does not inhibit HAF cell growth. The SEM observations revealed that more clusters of HAF cells were attached to the pore walls of both the PDLLA/5BG foam and the PDLLA/30BG foam when compared with the PDLLA/0BG foam. WST-1 assay performed over a period of 4 weeks showed an increased tendency of HAF cells to proliferate within both the PDLLA/5BG foam and the PDLLA/30BG foam when compared with both the tissue culture plastic control and the PDLLA/0BG foam, indicating the presence of Bioglass[®] in the foam has a positive effect on HAF cell proliferation. Sircol[™] and DMMB assays showed that HAF cells cultured within the PDLLA/30BG foam had a greater ability to deposit collagen and proteoglycan when compared with the control and the PDLLA/0BG foam after 4 weeks in culture, suggesting that the increase of Bioglass[®] content may induce microenvironmental changes which promote the production of extracellular matrix containing abundant collagen and s-GAG. The immunohistochemical analysis of collagen production demonstrated that collagen produced in all cultures was predominantly of type I. These findings provide preliminary evidence for the use of PDLLA/Bioglass[®] composite as cell-carrier materials for future treatments of the intervertebral disc with damaged AF region.

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1. Introduction

The intervertebral discs (IVDs) link adjacent vertebrae within the cervical, thoracic and lumbar spine. They are fibrocartilaginous in nature [1], and provide the spine with

its flexibility. The IVDs are composed of three major structural regions – the central nucleus pulposus (NP, a soft gel-like tissue), the inner annulus fibrosus (AF, both fibrous and cartilage like) and the outer annulus fibrosus (ligamentous) [2]. Each region contains an abundant extracellular matrix (ECM) surrounding cells with distinctive morphologies.

The origin of lower back pain is often damage to IVD due to factors such as injury, poor disc nutrition, vascular

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ingrowth and disc degeneration. The frequencies of back pain, stiffness and IVD degeneration change with age [3]. Common findings in degenerative disc disease include reduced proteoglycan content [4], diminished water content, a reduction in the number of cells within the NP and damage to the AF [5]. These changes may lead to alterations in disc shape and volume. Although disc degeneration is a normal physiological process, frequently it results in symptomatic spinal disorders, including pain, deformity and neurological compression. This process is therefore an active focus of research.

Current surgical treatments of IVD degeneration, such as discectomy and spinal fusion, although fairly successful in relieving back pain in the short-term, fail to restore the normal biological and mechanical properties of the human spine. The post-surgical problems associated with these treatments and their low clinical success rates are additional causes for concern [6].

New approaches are emerging for the treatment and cure of lower back pain as alternatives to current surgical procedures. These approaches, such as total disc replacement and nucleus replacement, aim to relieve back pain and restore the normal biomechanical motion of the human spine. Total disc replacement aims to replace the whole degenerated lumbar disc with an artificial structure. Because of the complexity of the disc structure, this approach requires a very sophisticated design of the artificial disc implant.

Annulus replacement by an artificial material or by tissue engineering is another potential approach for the treatment of back pain. Annulus replacement aims to replace only the degenerated annulus, keeping the remaining disc structure intact. However, this method is still in its infancy. Very few groups have tried to use this approach to replace the annulus, and the success has been only moderate [7–9]. In recent years, several investigations have been carried out for regeneration of IVD. Recent developments include growth factor treatments [10], gene therapy [11,12] and tissue engineering scaffolds [7,13,14].

Growth factors are polypeptides that modulate cell growth differentiation and matrix production [10]. The regulators of the IVD include insulin-like growth factor-1 (IGF-1), transforming growth factor- β (TGF- β), basic fibroblast growth factor (bFGF) and the bone morphogenetic proteins (BMPs) [15]. Based on this research, the direct injection of growth factors into the IVD was suggested as a clinical treatment for IVD degeneration. However, the dose required and the frequency and mode of delivery need to be further investigated. Gene therapy treatments involve the transfer of genetic material into a target cell to replace a defective sequence, to modulate the production of a beneficial substrate or ECM [11,12]. Genes which had been used include genes for growth factors, cellular regulators and tissue inhibitors of matrix metalloproteinase (TIMP). Although gene therapy is able to beneficially modulate IVD cells, there are safety concerns about the viral vectors used, which need further

investigation. Many materials have been investigated for a tissue engineering approach to repair or regeneration of the IVD and include calcium phosphate [16], collagen [17,18], fibrin [19], alginate [20], collagen–glycosaminoglycan (GAG) [21], collagen hyaluronan [13] and alginate [22] materials.

In this study, we chose to generate AF tissue on composite foam scaffolds made of poly(D,L-lactide) (PDLLA) with added Bioglass[®] particles because the scaffold degradation rate and the pore morphology can be controlled, and the scaffold's mechanical properties and structural integrity can also be improved [23,24]. Furthermore, it has been reported that PDLLA/Bioglass[®] composites exhibit higher *in vitro* bioactivity than equivalent composites which use hydroxyapatite as the bioactive phase, due to the intrinsic higher bioactivity of Bioglass[®]. Previous studies have shown that PDLLA/Bioglass[®] scaffolds have good biocompatibility with a variety of cells, including human osteoblasts, Schwann cells, human epithelia-like lung carcinoma cells, human osteosarcoma cells and bovine annulus fibrosus cells [25–28].

The fabrication of highly porous PDLLA and Bioglass[®] composite foam scaffolds were made using the thermally induced phase separation (TIPS) process, which has been reported previously [29,30]. The scaffolds have an anisotropic tubular pore morphology and extensive pore interconnectivity. The microporosity of TIPS-produced foams and their pore morphology, mechanical properties, bioactivity and degradation rates can be controlled by varying the polymer concentration in solution, the quenching temperature and the polymer and solvent used [31]. Bioactive glass, on the other hand, contributes to the mechanics, bioactivity and degradation kinetics of the foams.

In the present study, we investigate the cell response to PDLLA foams containing different concentrations of Bioglass[®] particles in *in vitro* conditions using human AF cells. Cell viability, attachment, proliferation and secretion of proteoglycan and collagen were performed on three different compositions of PDLLA/Bioglass[®] foams (PDLLA containing 0, 5 and 30 (wt.%) Bioglass[®]).

2. Materials and methods

2.1. Foam preparation

PDLLA/Bioglass[®] composite scaffolds were kindly supplied by Jonny J. Blaker, Department of Materials and Centre for Tissue Engineering and Regenerative Medicine, Imperial College London. The PDLLA used was Purasorb[®] PDLLA with an inherent viscosity of 1.62 dl g⁻¹ (obtained from Purac Biochem, Goerinchem, The Netherlands). Dimethyl carbonate (DMC, 99% in purity) (Sigma Aldrich, UK) was used as a PDLLA solvent in the fabrication process. The Bioglass[®] used was melt-derived 45S5 grade powder supplied by Novamin Technology Incorporated (Alachua, FL). The powder had a mean particle size of 5 μ m, a density of 2.825 g cm⁻³, a surface area of

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