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Open porous microscaffolds for cellular and tissue engineering by lipid templating

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ABSTRACT

Porous microspheres fabricated from biodegradable polymers have great potential as microscaffolds in tissue engineering applications, especially for novel strategies such as microtissue fabrication *in vitro* and microtissue assembly *in vivo*. Fabrication techniques for microparticulate scaffolds with surface and bulk pore sizes relevant for effective cell intrusion, however, are scarce. This study presents two techniques for the fabrication of open porous microscaffolds from poly(lactide-*co*-glycolide) in which lipid templating is used for pore formation and combined with either dispersion spraying or a double emulsion technique to determine the size and shape of the particulate structures generated. Both techniques yield microscaffolds with an average size of between 500 and 800 μm, high bulk porosities and open surface pores larger than 50 μm in diameter. Microscaffold morphology was investigated microscopically, particle size distribution was determined and porosity was quantified by intrusion measurements. Particle size and morphology was controlled by the processing parameters and the content and type of lipid porogen. Efficient extraction of the lipid template was shown by thermal analysis. Microscaffold cytocompatibility and *in vitro* cell culture performance was evaluated with L929 fibroblasts and rat adipose-derived stromal cells (ADSC), respectively. Extracts of different formulations were cytocompatible. Rat ADSC proliferated on the microscaffolds and were differentiated along the adipogenic lineage.

1. Introduction

Technologies that involve the proliferation and differentiation of isolated mammalian cells in a tissue-like 3-dimensional (3-D) environment *in vitro* have fostered fundamental advances in a wide variety of fields, including cell biology, biotechnology, drug development, cancer research and regenerative medicine [1–4]. Significant advantages of 3-D cell cultures over classical monolayer cultures have been identified regarding cell–cell interactions, cell–extracellular matrix interactions, cellular differentiation, metabolic activity and construct biomechanics [2,5]. In order to allow the 3-D cultivation of anchorage-dependent cells macroporous solids, fiber meshes and hydrogel matrices have been developed from numerous sorts of biomaterials in various geometries and used as permanent or temporary 3-D supports, so-called scaffolds [6–8]. The most popular techniques for the fabrication of macroporous tissue engineering scaffolds, particularly from biodegradable polymers, include solid free-form fabrication, electrospinning, freeze drying and porogen leaching techniques [9]. Among these processes, porogen leaching techniques are probably the most versatile and allow convenient control of pore size, geometry and interconnectivity in easy to use, laboratory scale processes [10].

One major challenge associated with the *in vitro* cultivation of cells in a 3-D context is to ensure a sufficient supply of nutrients, especially oxygen, to the cells throughout the volume of the construct and effectively remove metabolic waste products [11–13]. Most strategies rely on transport by passive diffusion. For such a process distances of not more than 300 μm can be adequately supplied in engineered tissue constructs *in vitro*. The use of bioreactors that actively perfuse cell–scaffold constructs during cultivation can significantly improve these supply problems [14,15], but are associated with additional instrumentation and a higher risk of contamination. With regard to the *in vivo* application of bioreactor cultivated tissue constructs, it has not yet been established how the engineered tissue volumes can be effectively connected to the host circulation upon transplantation in order to maintain construct perfusion and survival. An alternative strategy to address these challenges has recently been proposed and focuses on the *in vitro* engineering of small volume, high quality tissue constructs for *in vivo* assembly into larger tissue constructs upon implantation [16]. Such an approach has several inherent advantages: the constructs can be engineered and supplied with basic cell culture equipment, the small volume tissue subunits can be transplanted using minimally invasive techniques and co-transplantation

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