JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 46 (2015) 331–342

Available online at www.sciencedirect.com

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Research Paper

Porous matrix of calcium alginate/gelatin with enhanced properties as scaffold for cell culture

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ARTICLE INFO

Article history:
Received 19 June 2014
Received in revised form
20 August 2014
Accepted 27 August 2014
Available online 3 February 2015

Keywords:
Alginate
Gelatin
Aerated porogen
Scaffold
High porosity
Cell culture

ABSTRACT

Hydrophilic polysaccharides can be used to prepare porous matrices with a range of possible applications. One such application involves acting as scaffolds for cell culture. A new homogeneous and highly porous biopolymeric porous matrix (BPM) of calcium alginate/gelatin was produced by following a simple process. The key to this process was the selection of the porogen (aerated gelatin). The preparation technique comprises the following steps: incorporating the porogen into the solution of alginate (3%), molding, cross-linking the alginate in 1.41% $CaCl_2$ (maximum gel strength; Cuadros et al., 2012. Carbohydr. Polym. 89, 1198–1206), molding, leaching and lyophilization. Cylinders of BPM were shown to have a relative density of 0.0274 ± 0.002, porosity of $97.26 \pm 0.18\%$, an average internal pore size of 204 ± 58 μm and enhanced mechanical properties, while imbibing more than 11 times their dry weight in water. In vitro cell culture testing within BPM using mesenchymal stem cells was demonstrated by MTT assays and expression of alkaline phosphatase. The BPM provided a suitable microenvironment for seeding, adhesion, proliferation and osteogenic differentiation of cells. The preparation technique and resulting porous matrix represent potential tools for future study and further applications.

1. Introduction

Porous matrices from biomaterials take the form of solid foams, sponges, clusters of air cells, cellular solids or scaffolds. Several biopolymers are used to generate porous matrices which included collagen (Chimenti et al., 2011), gelatin (Chimenti et al., 2011; Liu and Ma, 2009; Sisson et al., 2010; Van Vlierberghe et al., 2007), silk (Jin et al., 2004), alginate (Alnaief et al., 2011; De Moura et al., 2005; Eiselt et al., 2000; Kaklamani et al., 2014; Ming-Hua et al., 2004), and chitosan (Geng et al., 2005; Ming-Hua et al., 2004).

Polysaccharides are widely used for their solubility in water, stability to pH variations and lack of toxicity (Barbosa et al., 2005). Alginate is a natural linear polysaccharide copolymer composed of two uronic acids units: β-(1,4) linked D-mannuronic acid (M) and α-(1–4) linked L-guluronic acid (G).

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http://dx.doi.org/10.1016/j.jmbbm.2014.08.026
1751-6161/

It contains varying quantities and sequences of three types of blocks (M–M, G–G, and M–G) and has the ability to form strong thermoresistant gels. These are produced by cross-linking with calcium ions within the G-block units to form local molecular arrangements known as "egg box" (Alnaief et al., 2011; Drury and Mooney, 2003; Grant et al., 1973; Sikorski et al., 2007; Simpson et al., 2004). This is performed under mild conditions at low temperatures and in the absence of organic solvents.

Alginate is widely used because of its non-toxicity, biodegradability and high biocompatibility (Kaklamani et al., 2014). Alginate gel is widely used in medical applications (Mikos et al., 2006; Ribeiro et al., 2004; Wang et al., 2008), such as tissue engineering (TE) (Petrenko et al., 2011). Gelatin is a protein derived from denatured collagen and is the major constituent of skin, bones and connective tissues. As a thermally reversible gelling agent, it can be used for encapsulation in food, cosmetics and pharmacology. It is also particularly useful in tissue engineering given its biodegradability, biocompatibility and non-immunogenicity (Wang et al., 2012; Yao et al., 2012). Calcium chloride is one source of divalent cations that has been widely used in biomedicine (Fan et al., 2005; Szymanski and Feinberg, 2014) for a long time, without any reports of calcium ions causing damage and/or affecting cell survival (Cao et al., 2012; Simpson et al., 2004). Calcium is preferred for applications in bone tissue as it is the principal ion in the extracellular matrix (ECM) (Morais et al., 2013). Furthermore, the calcium ion is both intra- and extracellular and has tremendous versatility as it is responsible for controlling several cellular processes such as: fertilization, proliferation, development, learning and memory, contraction and secretion (Berridge et al., 2000).

In general, high-G alginates produce strong but brittle gels, while high-M alginates produce weaker, more elastic and freeze/thaw stable gels (Sriamornsak et al., 2007). It has been shown that the strength of the alginate gel network is an important factor that influences the growth characteristics of encapsulated cells. While changes in gel strength affect the growth characteristics of the cells, high-M alginates are not sensitive to such changes (Simpson et al., 2004). The effect of the concentrations of $CaCl_2$ on the mechanical properties of calcium alginate fibres was studied; in it the maximum tensile stress was determined and was produced with a 1.41% (w/v) or 127 mM concentration of calcium chloride (Cuadros et al., 2012). A similar concentration of $CaCl_2$ (100 mM) has been used for two decades as a standard protocol for encapsulation and no toxic effects have been reported (Simpson et al., 2004).

To mimic the biological functions of native extracellular matrices, highly biocompatible scaffolds that promote cell adhesion and growth are essential (Leong et al., 2003). Besides biocompatibility, scaffolds must also demonstrate high porosity and interconnectivity of pores in order to promote cell seeding and growth. This is because the regeneration of tissues requires different microenvironments with suitable pore sizes. For TE cell in-growth and improvement of the transportation of nutrients requires porosities higher than 90% (Freyman et al., 2001; Leong et al., 2003). In terms of pore size, a range between 200 and 400 μm has been suggested for in vitro bone tissue regeneration (Leong et al., 2003), while another study recommends sizes between 300 and 500 μm (Hutmacher, 2000). The matrix must also have sufficient mechanical integrity during in vitro cell culture to maintain the spaces required for cell in-growth and tissue development (Drury et al., 2004). Mesenchymal stem cells (MSCs) are primitive multipotent cells that are able to differentiate various cell types. They can easily be isolated and propagated in vitro and are therefore suitable for bone TE as they exhibit two important properties: self-renewal and multi-lineage differentiation (Eslaminejad et al., 2006; Heng et al., 2004).

Several manufacturing techniques have been developed to confer porosity and homogeneity to porous matrices in order to improve the uptake, distribution and transport of different fluids and their components. There are many top-down techniques, including porogen-leaching (Studenovská et al., 2008; Weng and Wang, 2001; Zhang et al., 2006), gas foaming (Eiselt et al., 2000), thermally induced phase separation (TIPS) (liquid–liquid demixing and solid–liquid demixing, freeze-fixation, freeze-gelation) (Cheng-Hsuan et al., 2007; Holzwarth and Ma, 2011; Liu and Ma, 2009; Van Vlierberghe et al., 2007; Zmora et al., 2002), and lyophilization (direct method). Lyophilization is normally included at the end of any procedure for manufacturing porous matrices, as it is a way of directly removing the water from the frozen system. However, these top-down techniques have limitations, such as lack of mechanical strength, problems with residual solvent, lack of control over microstructure, presence of toxic residual porogens, nonporous external surface, close pore architecture, limited interconnected pores, small pores sizes or highly irregular pores, besides being tedious and time-consuming to fabricate (Leong et al., 2003; Martynov et al., 2010; Yao et al., 2012). For example, in freezing methods such as fixation by freezing and freeze-gelation, the architecture of the air cells showed elongated pores, the size of which differed from the top to the bottom of the matrix (Cheng-Hsuan et al., 2007; Ming-Hua et al., 2004). Producing biocompatible scaffolds for TE requires structures that have suitable macro-properties such as spatial form, mechanical strength, density and porosity, and micro properties such as pore size, pore size distribution and interconnectivity (Leong et al., 2003).

This study expects to overcome most of the limitations of the literature. The authors propose the development of a top-down production method resulting in a structure that is suitable for TE. The suitability of the structure is defined by both the macro properties as spatial form, strength, density and porosity homogeneous scaffolds; and micro properties such as pore size and interconnectivity. Therefore, the objective of this study is to develop a top-down technique for preparing a biopolymeric porous matrix (BPM) made primarily of alginate. A further objective was to determine the physical, mechanical and microstructural properties of the BPM. In vitro tests were also conducted to demonstrate the adhesion, growth and cell differentiation within the porous matrix.

2. Materials and methods

2.1. *Materials*

Sodium alginate (Alg) powder (Gelymar, Natural Extracts S.A., Chile) from *Macrocystis pyrifera* with an average composition