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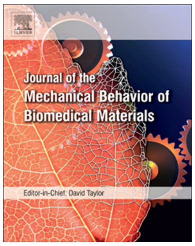

Research Paper

Mechanical properties of alginate hydrogels manufactured using external gelation

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ABSTRACT

Alginate hydrogels are commonly used in biomedical applications such as scaffolds for tissue engineering, drug delivery, and as a medium for cell immobilisation. Multivalent cations are often employed to create physical crosslinks between carboxyl and hydroxyl moieties on neighbouring polysaccharide chains, creating hydrogels with a range of mechanical properties. This work describes the manufacture and characterisation of sodium alginate hydrogels using the divalent cations Mg^{2+}, Ca^{2+} and Sr^{2+} to promote gelation via non-covalent crosslinks. Gelation time and Young's modulus are characterised as a function of cation and alginate concentrations. The implications of this work towards the use of environmental elasticity to control stem cell differentiation are discussed.

1. Introduction

Hydrogels have been extensively used in biomedical and biomaterials applications, for example as scaffolds for tissue engineering, biosensing and drug delivery technologies, and cell immobilisation (Khademhosseini and Langer, 2007; Fedorovich et al., 2007; Nicodemus and Bryant, 2008; Jen et al., 1995; Hoffman, 2002). There is significant interest in their use as matrices for tissue engineering, with both naturally occurring, synthetic, and hybrid materials affording the ability to specify both mechanical and chemical properties. Cells exhibit the ability to sense the mechanical properties of the environment with which they are in contact (Discher et al., 2005). Thus the elastic modulus of polymer hydrogels can influence their migration, development and differentiation (Peng et al., 2011; Engler et al., 2006). Hydrogels

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also serve as mimics for the extracellular matrix (ECM), which is known to influence the adhesion, geometry and proliferation of cells within their environment (Shoichet et al., 1996; Brandl et al., 2007; Banerjee et al., 2009).

Alginate is a naturally occurring polysaccharide derived from brown algae. It is an unbranched block copolymer composed of the two glycan monomers β-D-mannuronic acid (ManA, or M) and α-L-guluronic acid (GulA, or G) (Augst et al., 2006). It is non-toxic, biodegradable and can be used to form hydrogels under cytocompatible conditions. Alginate hydrogels have been used in bone and cartilage tissue engineering (Alsberg et al., 2001; Stevens et al., 2004), as vehicles for cell delivery, wound dressings, and as matrices to immobilise cells (Mooney and Lee, 2001; Drury and Mooney, 2003). They are commonly formed via ionotropic gelation of dissolved alginate in the presence of multivalent cations such as Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} (Morch et al., 2006; Topuz et al., 2012). Delivery of the cations into the alginate solution is often performed using an emulsification method, leading to the production of alginate hydrogel beads; this is usually described as internal gelation (Chan et al., 2002a, 2002b). In contrast, alginate hydrogels can be gelled externally via diffusion of cations from a porous solid support in contact with an alginate solution (Hunt et al., 2010).

The mechanical properties of alginate hydrogels may be tailored through variation of (i) the number and sequence of ManA and GulA monomers, and (ii) the concentration of the polymer in solution. Alginates with high GulA content tend to yield hydrogels with greater mechanical stiffness and strength than those with high ManA content (Drury et al., 2004). The elastic modulus depends on the number density of physical crosslinks between chains, conferred by the presence of cations (Banerjee et al., 2009). Alginate hydrogels formed with slower rates of gelation tend to exhibit greater structural homogeneity and therefore larger modulus than those gelled rapidly (Kuo and Ma, 2001).

The current work characterises the mechanical properties and gelation rate of externally gelled alginate hydrogels, prepared using porous microcellulose sheets as boundary materials. The sheets are separated by a layer of aqueous sodium alginate solution, similar to the methodology previously employed by Hunt et al. (2010) for the manufacture of 20 mm diameter discs. In contrast, in the work presented here the gelled discs are >100 mm in diameter, prepared using poly(styrene) supports to hold the microcellulose sheets in place. The sheets are saturated with an aqueous solution of divalent cation chloride that diffuses into the alginate solution, the cation acting as a crosslinking agent between the carboxyl groups of the alginate molecules. Group 2 cations Mg^{2+}, Ca^{2+} and Sr^{2+} are investigated at solution concentrations in the range 1–5 M. The cations Be^{2+} and Ba^{2+} were not investigated because of their toxicity, whilst Ra^{2+} was not investigated because of its radioactivity. The rate of gelation is assessed and the mechanical properties of the resultant hydrogel are measured using indentation. Furthermore, acellular culture media and 3T3 fibroblast-containing culture media are used as a solvent for the sodium alginate and the mechanical properties of these gels are also measured.

External gelation affords the possibility of creating large samples of uniform thickness, with a range of geometries and good reproducibility. The geometry chosen here is a thin disc-like cylinder, formed between parallel platens. The sample exhibits a surface with roughness similar to that of the microcellulose sheet. Further, the samples manufactured in this work are of a geometry suitable for reliably measuring mechanical properties using the spherical indentation technique outlined in Section 2.5. Samples gelled in this way can also be easily cut to shape for subsequent use. External gelation offers a more rapid gelation than internal gelation, which can typically take up to 48 h. For example the $CaSO_4$/D-glucono-δ-lactone/$CaCO_3$ system is reported by Kuo and Ma (2001), which requires dispersal of $CaCO_3$ particles within the viscous pre-gelled matrix; a uniform distribution is difficult to achieve, leaving the possibility of local fluctuations in material properties and residual undissolved solid particles within the gel. In contrast, gel beads can be formed rapidly by dripping alginate solution into an aqueous solution of Ca^{2+}, which does not give concern for residual undissolved material. However, this method often results in a polymer concentration gradient within the bead (Skjåk-Bræk et al., 1989).

2. Experimental

2.1. Materials

All chemicals were sourced from Sigma-Aldrich (UK) unless otherwise stated. Purities were >99% in all cases. HPLC grade H_2O was employed throughout.

2.2. Hydrogel manufacture

Hydrogels were prepared by an external gelation method in which aqueous sodium alginate solution was poured into a poly(styrene) mould (141.4 mm inside diameter, 9.0 mm inside height, Sterilin, UK) to a liquid height of 6 mm and allowed to gel in the presence of an aqueous solution of cation chloride held at the upper and lower boundaries by porous microcellulose sheets. Prior to the addition of the aqueous sodium alginate solution, stainless steel cylindrical spacers (21 mm diameter, 6 mm height, Longshore Systems Engineering, UK) were placed at 60° intervals around the inner edge of the mould in order to support the upper sheet. The volume of aqueous sodium alginate solution required was 81.75 mL. The upper sheet was held in place from above using a poly(styrene) support, filled with water in order to maintain close contact between the upper sheet and the sodium alginate solution as it gelled since some shrinkage was observed at the sample edges. A schematic of this process is shown in Fig. 1.

Sodium alginate powder was dissolved in H_2O under agitated conditions at a temperature of 70 °C and stirred for a minimum of 2 h using a Stuart US-152 hotplate-stirrer (Appleton Woods, UK). Sodium alginate solutions of concentrations in the range 2.5–5.0% (w/v) were prepared. Aqueous cation chloride solutions of concentrations in the range 1–5 M, where solubility permitted, were created by gradually dissolving the cation chloride powder in water under agitated conditions at room temperature, which in this case was 18 °C; the dissolution was usually exothermic and hence care was