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Fabrication of alginate fibers using a microporous membrane based molding technique

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ABSTRACT

Tubular structures made of alginate and similar hydrogels have been extensively investigated for drug delivery, tissue engineering and other biomedical applications. Alginate fibers are usually fabricated using complicated techniques such as coaxial flow extrusion or electro-spinning. This paper discusses the fabrication of hollow and solid alginate fibers using a simple membrane based molding technique. A hollow-fiber microfiltration membrane served both as the mold as well as reservoir for the cross-linking agent. The pores of the membrane were first filled with the cross-linker (i.e. calcium chloride) solution, after which the lumen was filled with sodium alginate solution. The calcium ions diffused from the membrane pores into the lumen, cross-linking the alginate in a radially inward direction. Solid alginate fibers were obtained by cross-linking all the alginate within the lumen, while hollow fibers were fabricated by pushing out un-cross-linked alginate from the central core using calcium chloride solution. The alginate fibers fabricated as described above were expelled from the membrane mold using water under pressure. These were then characterized by optical, fluorescence and scanning electron microscopy. The most attractive features of this fabrication method are its simplicity and flexibility with regards to alginate concentration. The fibers obtained were straight with excellent definition and uniform thickness, and could be fabricated in a highly reproducible manner. Other complicated solid and hollow 3-dimensional structures suitable for biomedical, and indeed other applications could also potentially be fabricated using similar membrane-based molding techniques.

1. Introduction

Hydrogels can hold large amounts of water within their structures and are widely used for a range of pharmaceutical and biomedical applications [1,2]. Synthetic hydrogels such as cross-linked polyethylene glycol (PEG), poly (N-isopropylacrylamide) (PNIPAM) and poly-hydroxyethylmethacrylate (PHEMA) can be tailored to specific chain lengths and degree of cross-linking, and can also be functionalized to make them stimuli-responsive, an attribute extremely useful for controlled or sustained release of drugs [3]. However, un-polymerized monomer residues can be highly toxic, and their complete removal can prove to be challenging. Consequently, natural hydrogels continue to be widely used for drug delivery, cell culture and tissue engineering applications, where toxicity due to monomers and cross-linkers could be a major issue [4,5]. Alginate, a polysaccharide obtained from brown algae is widely used for cell encapsulation, drug delivery, wound-healing, and tissue engineering application [6–8] and is generally regarded as safe (GRAS). The gelation of alginate is induced by the presence of divalent cations such as, Ba^{2+}, Sr^{2+}, and Ca^{2+} which interact with the G residues [6,7]. Due to the involvement of the G residue in cross-linking, the G/M ratio affects the mechanical strength and biodegradability of alginate [7,8]. Spherical calcium alginate beads have been widely used for cell encapsulation and drug delivery due to the simple fabrication method, i.e. drop-wise addition of sodium alginate solution into cross-linker (calcium chloride, $CaCl_2$) solution [7]. The material to be encapsulated is typically pre-mixed with the sodium alginate solution prior to cross-linking. Some of the drawbacks linked with spherical alginate beads and their fabrication method are (a) loss of cells or drug into the cross-linking solution, (b) the so-called "burst-effect" whereby there is an initial rapid release of drug from the beads to any recipient solution, and (c) nutrient mass transport limitations at the center of the beads which could lead to cell necrosis [9–12]. The drop-wise bead production methods also lead to size distributions, and this makes precise drug dosing difficult. A critical limiting factor for the spherical geometry is that the surface area to volume ratio is determined by the radius, thereby limiting flexibility in designing structures.

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Alternative geometries such as tubes, rods and cylinders have been shown to have some advantages in certain drug delivery and tissue engineering applications [13]. With the cylindrical form, dosage can be very precisely controlled based on length and diameter. Also, the surface area to volume ratio is decoupled from the radius and can be flexibly manipulated by altering both the radius and length of the structure. Moreover, in certain applications such as nerve and vascular tissue engineering, the fiber is the only suitable scaffold geometry.

Electro-spinning, one of the well-established methods for fabricating fibers, has attracted much attention due the ease with which fine fibers with diameters ranging from 10 nm to 100 μm can be produced using both synthetic and natural hydrogel materials [14–19]. However, this technique involves the application of high voltages, e.g. 10–15 kV [17], 20 kV [18], and 13–15 kV [19], which could potentially lead to cell mortality in cell immobilization and tissue engineering applications. During cell-embedded fiber fabrication by electro-spinning, cell death was as high as 60% [20]. Moreover, electro-spinning is not particularly suitable for fabrication of fibers with outer diameter greater than 100 μm [17,19,21]. Another major problem with electro-spinning of alginate is that the method is restricted to the use of alginate solutions of lower than 2% concentration due to viscosity issues, and consequently fibers with low mechanical strengths are produced [21].

Extrusion based technique have also been widely used to produce alginate fibers. For instance, Li et al. [11] reported a very simple technique for producing alginate cylinders by directly injecting sodium alginate into $CaCl_2$ solution. Improved versions of this technique involving sophisticated multichannel nozzles, suitable for fabricating more complicated structures have been subsequently reported [22–24]. These techniques were suitable for producing 100–800 μm diameters fibers by manipulating variables such as reagent concentration and extrusion velocity, and by using sheath solutions [25]. The combinations of extrusion and microfluidic techniques have been shown to enhance the consistency of fiber production [26–28]. However higher concentrate alginate solutions cannot be satisfactorily handled by microfluidic systems as their high viscosity makes it difficult to balance the central flow and sheath flows, which in turn leads to non-uniformity in fiber dimension. Moreover the high shear stress observed in such system could be problematic in cell immobilization and tissue engineering applications. In an effort to reduce shear stress, a roller assisted microfluidic system was developed whereby alginate fibers produced within microfluidic channels were drawn out in a controlled manner [29]. Other techniques reported for fabrication of alginate fibers include 3D printing [30], laser-assisted printing [31] and the use of centrifugal force [32]. Boland et al. [33] have reviewed the use of 3D printing in tissue engineering including the fabrication of alginate structures.

In this paper, we describe a molding technique, using which hollow and solid alginate fibers can be fabricated under gentle and sterile conditions. A hollow-fiber micro- or ultra-filtration membrane is used as the mold for fabricating these alginate fibers. The pores present on the hollow fiber membrane served as the reservoir for $CaCl_2$ solution was used to cross-link alginate within the fiber lumen. The method is summarized in Fig. 1. As shown in A, the pores of the hollow fiber were first filled with $CaCl_2$ solution. The lumen of the hollow fiber was then filled with sodium alginate solution (B). The divalent calcium ions diffused from the pores into the lumen, cross-linking the alginate in a radially inward direction (C). For producing solid fibers, the alginate held within the lumen was allowed to fully cross-link before expelling it from the hollow-fiber membrane. For producing alginate hollow fibers, the cross-linking was allowed to take place till the desired wall thickness was attained followed by removal of uncross-linked alginate from the interior core using $CaCl_2$ solution (D). This step also resulted in the curing of the inner surface of the alginate hollow fiber, which was then recovered by expulsion from the mold under pressure using water or $CaCl_2$ solution (Fig. 1E). Alginate fibers of different sizes and geometries were fabricated using the above technique. Effects of variables such as alginate concentration, cross-linker concentration and cross-linking time on the fiber dimension were examined to demonstrate the controllability of the fabrication technique. The alginate fibers produced were tested using techniques such as scanning electron microscopy (SEM), light microscopy and fluorescence microscopy. The feasibility of using the molding techniques for tissue engineering and cell immobilization applications was demonstrated by fabricating alginate hollow fibers containing live human umbilical vein endothelial cells (HUVEC).

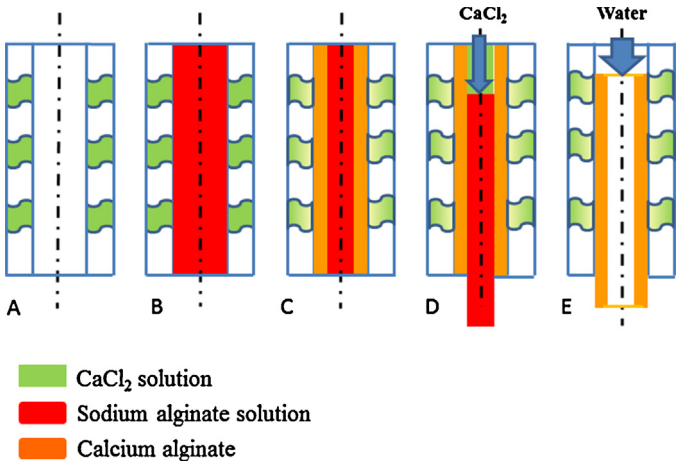

Fig. 1. The method for fabricating calcium alginate hollow fibers using porous hollow fiber membrane as mold (A) cross sectional view of hollow fiber membrane with pores filled with $CaCl_2$ solution, (B) sodium alginate solution being filled inside the lumen of the hollow fiber membrane, (C) cross-linking of alginate by radially inward diffusion of calcium ions, (D) removal of un-cross-linked alginate core with $CaCl_2$ solution, and (E) expulsion of calcium alginate hollow fiber from mold.

2. Materials and methods

2.1. Materials

Sodium alginate (W201502, loss on drying 12.3%, arsenic <1 ppm, cadmium <1 ppm, mercury <1 ppm, lead <1 ppm), and FITC-dextran (46947; excitation 490 nm, emission 520 nm) were purchased from Sigma–Aldrich, St. Louis, MO, USA. Calcium chloride (C77-500) was obtained from VWR, Mississauga, ON, Canada. Polypropylene hollow fiber microfiltration membranes (Accurel PP S6/2, 1.8 mm i.d., 2 mm o.d., 0.2 μm pore size; Accurel Q3/2, 0.6 mm i.d., 1 mm o.d., 0.2 μm pore size) were purchased from Membrana, Wuppertal, Germany. These membranes have residual oil content of less than 100 ppm. Hydrophilic polyethersulfone hollow fiber ultrafiltration membranes (Tetronic, 0.8 mm i.d., 1.2 mm o.d., 150 kDa MWCO) was kindly donated by Hydranautics Inc., Japan. All test solutions and reagents were prepared using ultrapure water (18.2 MΩ cm) obtained from a Diamond Nanopure water purification unit (Barnstead International, Dubuque, IA, USA), vacuum-filtered just prior to use using 0.45 μm pore size cellulose acetate membrane (Nalgene Nunc, Rochester, NY, USA).

2.2. Fabrication of calcium alginate hollow fibers

Membrane fabrication was carried out at room temperature (i.e. 22 °C). Sodium alginate solutions of different concentration (e.g. 1, 2.5, 5 wt.%) were prepared in ultrapure water, microfiltered through 0.22 μm membrane. The solutions were allowed to stand