

Fabrication of Porous Gelatin Microfibers Using an Aqueous Wet Spinning Process

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Abstract: Alginate has a unique property of gel formation by chelating with divalent cations such as Ca^{+2} in aqueous solution. The sol-gel characteristic of alginate has been utilized to fabricate both microsphere and microcapsule for cell immobilization. In this study, a wet spinning process was employed to prepare fibers comprised of gelatin and sodium alginate. Gelatin fibers containing interconnected porous structure were fabricated by the extraction of alginate from these gelatin/alginate composite fibers with phosphate buffer. The application of these porous gelatin fibers for enzyme immobilization was demonstrated by its usage as the base material of glucose biosensor.

Keywords: gelatin, alginate, fiber, glucose sensor

INTRODUCTION

Gelatin, a biodegradable polymer derived from partial hydrolysis of denaturing collagen, has collagen-like chemical composition and biocompatibility. Because of its biological similarity to collagen and commercial availability at relatively low cost, gelatin has been used in biomedical devices such as sealants for vascular prostheses [1,2], carrier for drug delivery [3,4], wound dressings [5], and bone grafts [6]. The gelatin-based biomaterials were presented in various forms [7–9] tailored to their specific needs but most commonly in the configurations of slab or porous sponge. For applications in protein immobilization and tissue engineering, the usage of polymeric microfibers is advantageous for higher seeding efficiency of enzyme and cells, and also the versatility of weaving into various shapes. Unfortunately, the technology development of gelatin fiber fabrication is hampered by its poor fiber-forming characteristics. Recently, gelatin has been successfully spun into nanoscale fibers by using an electrospinning technique. The application of e-spun gelatin fibers is rather limited, mainly for non-woven meshes. On the other hand, fibers prepared from the wet spinning process can be easily woven into various forms in addition to the formation of non-woven mesh. Unfortunately, the fiber drawing process of gelatin is rather difficult and rarely investigated. Recently, a wet

spinning process to prepare gelatin microfibers has also been reported by using dimethyl sulfoxide as a coagulant [10]. This method of fiber spinning involves the use of organic solvent, which imparts a major concern because of its residual toxicity.

Alginate, a polyanionic copolymer consisting of two hexuronic acid residues, β -D-mannuronic acid unit and α -L-guluronic acid, in varying proportions, can form stable gels at room temperature with calcium ions via ionic interaction with the guluronic acid residues [11]. Alginates have been long used for gelation of forming microsphere in aqueous environment [12]. By applying this concept, we here developed the technology of gelatin fiber fabrication via the formation of gelatin/alginate thread and the extraction of alginate to obtain porous structure gelatin microfibers. This technique could be applied to immobilize enzyme and bioactive molecules that are sensitive to organic solvents.

MATERIALS AND METHODS

Materials

Sodium alginate (mole wt 80000–120000) and gelatin type A (Approx. 300 Bloom) of porcine skin and glucose oxidase (type II-S, 35600 unit/g) were both purchased from Sigma (St. Louis, MO, USA). Acetic acid and an

This work was supported by a grant from National Science Council, Taiwan, Republic of China (NSC 97-2221-E010-007).

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aqueous glutaraldehyde solution (25%) were obtained from Merck (Hohenbrunn, Germany). All chemicals used in this study were of reagent grade.

Methods

Wet spinning process. An aqueous gelatin solution (2.45 mg/ml) was prepared by dissolving gelatin in double de-ionized water at 60°C. Sodium alginate was added to the aqueous gelatin solution with concentration adjusted to 1.5 wt%, and the mixed solution was stirred at room temperature with a magnetic stirring bar. The mixture was degassed for 30 min using a water pump. The gelatin/alginate mixture was then loaded in a syringe and extruded at room temperature from a spinneret (0.51 mm inner diameter) into a coagulation bath containing an aqueous solution of 2 wt% CaCl_2 (Figure 1). The solution extrusion speed and the fiber collection speed were controlled at 117 mm/sec (V_1) and 369 mm/sec (V_2), respectively, to attain a fiber draw ratio ($\text{DR} = V_2/V_1$) of 3.14.

Determination of water content. The gelatin/alginate wet spinning fibers were washed with double de-ionized water, and the fibers' diameter were measured under an optical microscope. The water content of gelatin/alginate wet spinning fiber was calculated by the equation:

$$(\text{W}_L - \text{W}_D)/\text{W}_L \times 100\%$$

where W_L and W_D are the weight of fully swollen fiber and dry weight, respectively.

Fabrication of gelatin fiber using the process of alginate extraction and cross linking. Alginate was extracted from the gelatin/alginate fibers by immersing the wet spun fibers in 0.25M PB at 4°C for 48 hours. The amounts of alginate released in the solution were measured with the carbazole method [13].

The chemical crosslinking was carried out by immersing gelatin fiber in a 0.25 M PB solution (pH 7.2) containing 2.5 wt% glutaraldehyde for 1 hrs. The

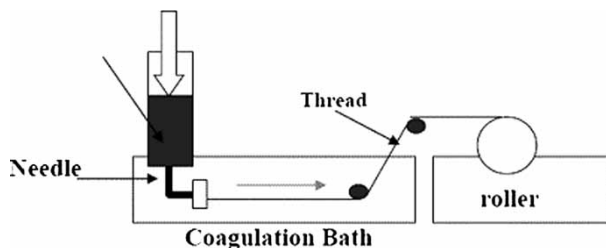


Figure 1. Apparatus of wet spinning process. An aqueous solution containing 2.45 mg/ml gelatin and 1.5 wt% alginate was loaded in the syringe and extruded into the coagulation bath (2% w/v CaCl_2).

gelatin fiber surface structure was dried with CPD and coated with gold before observed by SEM.

Construction of glucose sensor. An enzyme glucose sensor can be constructed conventionally by the following way. The graphite powder (60 mg) was first mixed with paraffin oil (21 μl) and packed into carbon-pasted electrode (0.95 mm diameter), leaving 1 ~ 2 mm space in depth for the matrix of sensor tip enzyme immobilization. Next, a mixture of graphite powder (20 mg), glucose oxidase (657 unit), and paraffin oil (7 μl) was packed into the remaining space of the electrode tip. When gelatin was used as an adding base material of the enzyme electrode, the gelatin wet spinning fiber (4 mg), graphite powder (20 mg), GOx (657 unit), and paraffin oil (7 μl) were mixed thoroughly and packed into the retaining space of the electrode.

Before electrochemical measurements, the solutions to be analyzed were deoxygenated by N_2 . Measurement of current at constant potential was carried out at room temperature on a three-compartment cell with an electrochemical work station BAS CV-50W by using an enzyme electrode as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire electrode as the counter electrode. Before measuring the electrochemical response of the enzyme electrode towards glucose, the electrode was immersed in a phosphate buffer solution (pH 7.4). Once the electrochemical system is stable, the catalytic current detected by the enzyme electrode will demonstrate a stepwise linear line due to the increase in glucose concentration.

RESULTS AND DISCUSSION

A typical bundle of gelatin/alginate fibers obtained by the present study is shown in Figure 2. The properties of these gelatin and alginate composite filaments (Figure 3) were investigated. In addition, gelatin fibers were prepared by extraction of alginate from the composite

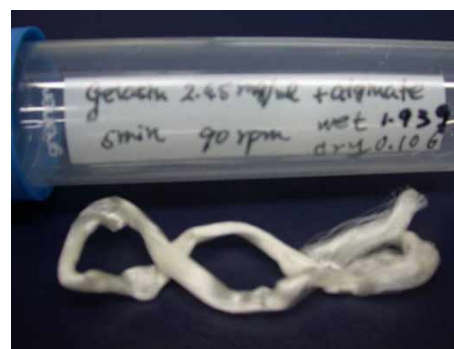


Figure 2. Photo of alginate/gelatin fiber obtained from wet spinning with draw ratio of 3.14.

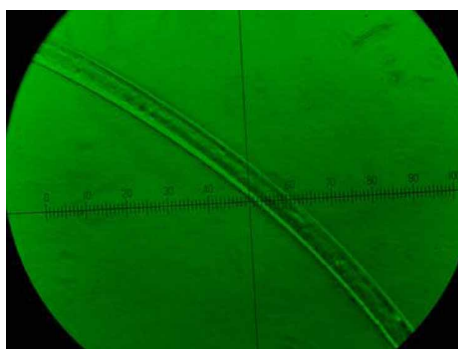


Figure 3. Wet spinning fiber of gelatin/alginate seen under optical microscope (from draw ratio of 3.14).

filaments and their application as the base material of glucose sensor was demonstrated.

The Effects of Roller Speed

The roller speed of the wet spinning apparatus affects the diameter and properties of the drawn fiber. The effect of the roller speed could be expressed in a parameter called draw ratio (DR: speed of roller/speed of injection from spinet). In this study, we have tested three different drawing ratios (DRs) of 3.14, 1.34 and 0 (non-drawing) to determine the effect of DR on fiber size. When the DR value was increased, the diameter of the drawn fiber decreased from 0.48 mm (non-drawing) to 0.24 mm (DR: 1.34) and 0.16 mm (DR:3.14). Beyond the DR value of 3.14, the gelatin/alginate fiber frequently broke and a continuous long fiber could not be obtained.

The gelatin/alginate as-spun fiber has a water content of about 94.3%, almost the same as that of alginate alone (95.6%).

Gelatin Fibers Obtained after Alginate Extraction with PB

The gelation of the alginate was via calcium chelation, and the alginate can be removed after being liquefied by

immersing in phosphate buffer (PB) at high concentration. We found that phosphate buffer at low concentration was incapable of liquefying the alginate. A phosphate buffer of at least 0.25 M could successfully remove the alginate. After being washed with 0.25 M PB buffer at 4°C for 48 hours, the alginate content in the alginate/gelatin spun fiber decreased substantially from 86% to 9.6%. This represents about 89% removal of alginate from the spun fiber and the extent of removal appears to be unaffected by either the draw ratio or the diameter of the fiber.

Because the strength of PB washed gelatin fiber was very low, it is therefore necessary to crosslink the gelatin fiber with glutaraldehyde at 4°C, a temperature under which the gelatin remained as an insoluble gel form. The crosslinked gelatin threads surface were examined by scanning electron microscopy. As estimated under SEM, the fiber diameters of dried gelatin after PB wash were about $181 \pm 2.1 \mu\text{m}$. As shown in Figure 4, there are many pores existing on the surface of gelatin fiber that is probably formed when alginate was leached out of the gelatin/alginate wet spun fiber. Using image-J software (<http://rsb.info.nih.gov/ij/>) to determine the porous structure, we found that the average pore sizes of the fibers was about $0.027 \pm 0.015 \mu\text{m}$ ($n = 60$).

Application of Gelatin Fibers as the Matrix for Enzyme Immobilization

One potential biomedical application of the gelatin fiber is its usage as the vehicle for enzyme immobilization, because the gelatin microfibers fabricated by the wet spinning process in this study have an interconnected nanoporous structure. It provides large surface for the physical absorption of enzyme. Figure 5 shows the advantage of using the gelatin fibers as the base materials of glucose sensor. As shown, the glucose sensor has higher sensitivity when gelatin fibers were used. The biosensor sensitivities were $0.85 \mu\text{A}/\text{cm}^2\text{mM}$ when gelatin fibers were included in the carbon paste. This sensitivity is

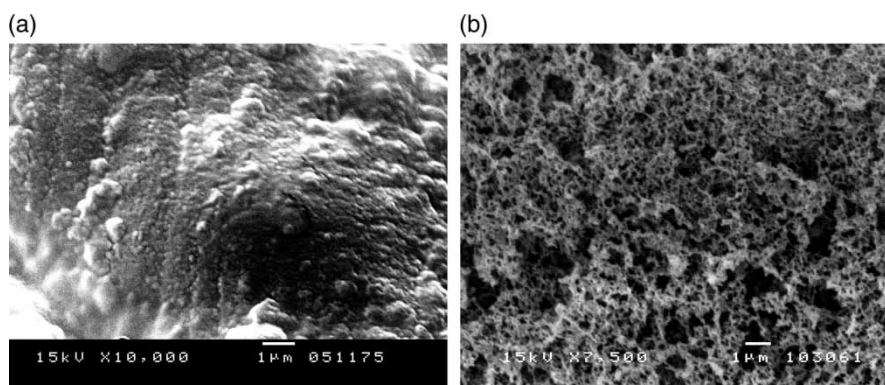


Figure 4. The surface structure of gelatin wet spinning fiber (draw ratio 3.14): (a) before, and (b) after alginate extraction.

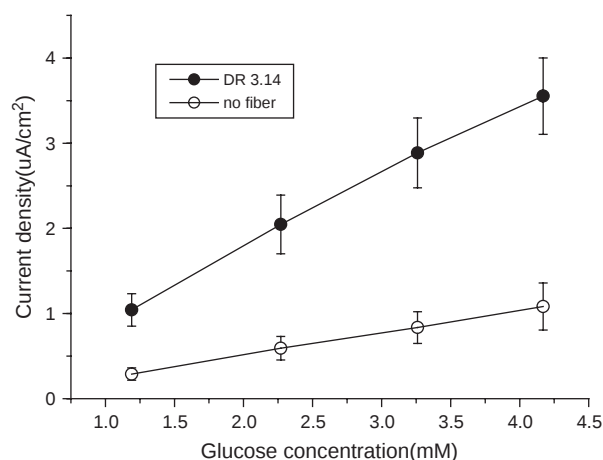


Figure 5. Comparison of the amperometric currents of glucose enzyme electrodes with and without gelatin fibers as base materials in responding to the various glucose concentrations. The biosensor sensitivities were $0.85 \mu\text{A}/\text{cm}^2\text{mM}$ (with gelatin fibers) and $0.26 \mu\text{A}/\text{cm}^2\text{mM}$ (without gelatin fibers).

more than three times the sensitivity ($0.26 \mu\text{A}/\text{cm}^2\text{mM}$) of the glucose sensor constructed conventionally without the addition of gelatin fiber. The porous gelatin fibers developed in this study could be used for immobilization of other biological active macromolecules.

CONCLUSION

We have developed an aqueous wet spinning process for the fabrication of porous gelatin fibers. These porous fibers have a great potential as the matrix for the immobilization of bioactive entities.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on iFirst on 12 June 2009.