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Novel control of gel fraction and enhancement of bonding strength for constructing 3D architecture of tissue engineering scaffold with alginate tubular fiber

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Alginate tubular fiber has been successfully prepared via coaxial fluid crosslink mode, which is potentially used for the construction of vascularized tissue engineering scaffolds (VTES). However, its elastic and smooth surface is negative for the adhesion of fibers. In this study, the gel fractions were controlled in a novel way of two-step crosslink process in order to meet the needs of each processing link. Based on such consideration, an appropriate formulation was selected to direct write single fiber, which ensured the tubular structure with enough gel portion as well as adhesion between fibers with the reserved sol. Finally, the integrity of the scaffolds had a further development within the 2nd crosslink bath process, which would help to solve the question of poor shear resistance for hydrogel scaffolds.

[**Key words:** Tubular fiber; Sodium alginate; Gel fraction; Interface bonding]

Alginate is considered one of the most popular biomaterials due to the number of characteristics: convenient sources, nontoxicity, excellent biocompatibility and biodegradability (1,2). Sodium alginate has a great potential for application in tissue engineering, pharmaceutical and clinical medicine as a carrier of drugs and cells (3).

One of the major challenges for in vitro engineered tissues is the difficulty creating engineered blood vessel systems, known as vascularized systems (4–6). There are reports of in vitro culture of large tissue-engineered constructs that can be sufficiently supplied with oxygen and nutrients by using perfusion bioreactors (7,8). However, after these tissues are implanted, the diffusion processes are limited due to the insufficient distances (9). Constructing the tissue engineering scaffold with vessel-like tubular fiber is a new attempt to solve the problem of vascularization in vivo (10–12).

Other than the traditional external gelation of hydrogel block, microsphere, solid fiber or membrane (13–16), the tubular fiber is derived from an annular alginate sol crosslinked from inside to outside, as seen in Fig. 1A. Lee et al. (17) established a coaxial fluids extrusion mode with the alginate sol inside of $CaCl_2$ solution in order to generate continuous solid hydrogel fibers. Hu et al. (18) reversed the position of the two solutions and added a $CaCl_2$ layer as the outermost to generate a tubular fiber on a coaxial triple-orifice microfluidic platform. The structure of coaxial triple-orifice spinneret was too complicated to guarantee concentricity, and greatly affected the uniformity of the tube wall. Ozbolat et al. (12) simplified the coaxial triple-orifice spinneret to a double-orifice style, which was also capable of generating a tubular fiber. The double-orifice spinneret (12,19) provided sufficiently dry process environment for the construction of the 3D tissue engineering scaffold architecture with the tubular fiber as depicted in Fig. 1B.

The previous studies attempted to make the annular alginate sol completely crosslink. However, it is harmful for VTES construction due to the fact that the resulted elastic and smooth surface is rather negative for fiber adhesion. Therefore, a novel control of the gel fraction in the manufacturing of hollow fiber and scaffold is proposed. A certain amount of sol was reserved to guarantee the adhesion between deposited fibers while direct writing. On one side, the cured portion is sufficient for supporting a tubular structure and unification of the uncured portion. On the other side, the reserved sol is capable of fusing the surfaces between overlapped fibers inhibiting fiber slip rapidly, while direct writing the 3D architecture of VTES, as depicted in Fig. 1C. Furthermore, the interface bonding strength in the state of sol and gel was examined aiming to develop the integrity of the hydrogel scaffold.

MATERIALS AND METHODS

Materials Sodium alginate (Chemical pure, molecular weight around 3.5×10^7 g mol^{-1}) and calcium chloride ($CaCl_2$) (Analytically pure, molecular weight 110.8) were used to fabricate the hydrogel fiber. Sodium alginate (3–5% w/v) was dissolved in deionized water and placed in a shaker for 10 h at 120 rpm at room temperature. Similarly, 2–4% (w/v) $CaCl_2$ solutions were prepared with deionized water. All reagents mentioned above were purchased from Sinopharm Chemical Reagent Co. (China).

Preparation of VTES The preparation of VTES was a typical 3D printing process. As shown in Fig. 1A, the process can be separated into three stages (α–γ). α stage. Sodium alginate sol was extruded through the shell section of the coaxial spinneret, while calcium chloride solution was extruded through the core section; β stage: Two fluids met, and crosslink was induced by the calcium ions diffusing

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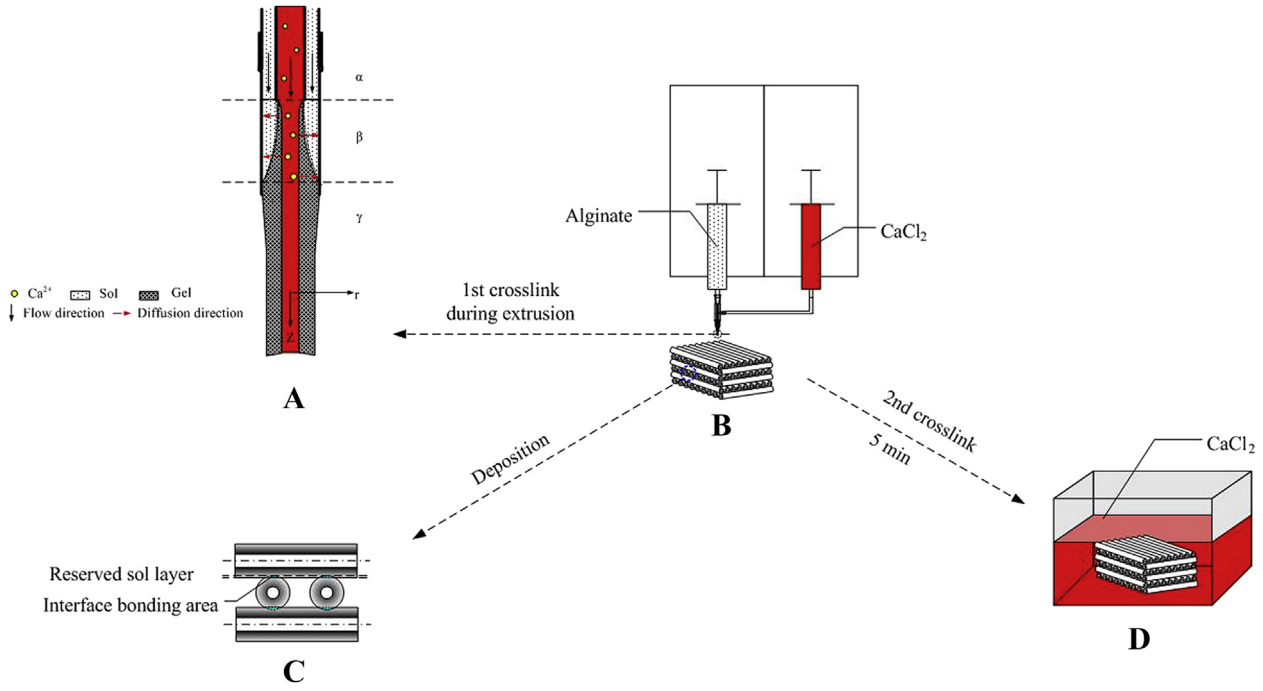

FIG. 1. Schematic illustration of the process flow of 3D printing VTES: (A) tubular fiber made from annular alginate sol crosslinked with CaCl2 solution; (B) 3D printing the architecture of VTES; (C) through the control of gel fraction, some sol was reserved to ensure the adhesion between fibers; (D) bonding strength was enhanced with crosslink bath.

from inner to outer in radial direction. Finally, the shell sol was cured completely to form a stable tubular structure; γ stage: guided by motion units, the tubular fiber was laid down to construct a scaffold with certain architecture.

In order to increase the interface bonding strength and make the scaffolds more integrated, the as-prepared VTES were immersed into 0.5% $CaCl_2$ crosslink bath for 5 min to cure the residual sol portion, especially for the sol at the bonding position (Fig. 1D).

A total of 12 kinds of formulations was carried out. In order to simplify the expression, corner mark was used to present the formulations. For example, $A_{4:3}$ represents 4% sodium alginate crosslinking with 3% calcium chloride. In order to meet the cell survival limits (11), the dispensing rates of sodium alginate and calcium chloride were set as 0.4 ml/min.

Pre-determination of gel fraction Based on the character that the residual sol exposed on the surface of the tubular fiber, the gel fraction was pre-detected using elution method (20,21). The details are as follows: (i) The extruded fiber was received in a Petri dish, then eluted with the magnetic stirrer (150 r/min, 60 min); (ii) The elution liquid and fiber were transferred into a 100 ml centrifuge tube, the dish and rotors were washed with deionized water thrice; also, the washing fluid was poured into the centrifugal tube; (iii) The separation between cured fiber and aqueous solution of residual sol was implemented by rapid centrifugation (3000 r/min) and filtration (pore size: 30 μm); (iv) Firstly, the filtrate was dried in a circulation oven at 60°C for 4 h and then transferred into a vacuum drying oven at 20°C for 10 h; Finally, for each formulation the weight of the dried alginate from five samples was collected and expressed in mean results. (v) The gel fraction G was calculated using weight method (22).

$$G = 1 - \frac{W_1 - W_0}{V_{Alg} \times C_{Alg}} \quad (1)$$

where C_{Alg} represents the concentration of alginate solution, $g \cdot ml^{-1}$; V_{Alg} represents the extrusion volume of alginate solution, ml; W_0 represents the quality of the empty Petri dish, g; W_1 represents the total quality of dried alginate and Petri dish, g.

Interface bonding strength The scaffold sample was simplified to the basic unit: a mode of two orthogonally overlapped fibers. The bonding strength was measured with a universal testing machine (Zwick/Roell and testXpert software). Each fiber of the sample was folded in half through the bonding point. Next, the two ends of the fibers were gripped with calipers. The tensile load was applied at a speed of 10 mm/min, temperature of 25°C, and relative humidity of 65%, until fracture occurred. Since the area of the fused interface was complicated for measurement in this test, the bonding strength was evaluated by detecting the tensile load while the two fibers separated, and expressed as Newton (N) (Fig. 2).

To obtain three kinds of representative gel fractions, the samples were prepared with three formulations ($A_{4:2}$, $A_{4:3}$ and $A_{4:4}$), regardless of the integrity of the tubular fiber. For each kind of formulations, five samples were bonding by the residual sol, and the other five underwent the 2nd crosslink. The mean bonding strength and standard deviation were calculated from the fracture loads.

Morphology of the tubular structure The morphology of the tubular fiber and scaffold was detected by optical microscope (EV3020, Easson, China), SEM (SU1510, Hitachi, Japan) and Micro CT (μCT 80, Scanco Medical, Switzerland).

In general, the performance of an optical microscope in reflecting the internal structure depends on the transmittance of the objects. In this study, for the purpose of sharpening the wall boundary, a section of air bubble was injected into the tubular fiber, as shown in Fig. 6A. For SEM detection, the tubular fiber were first dehydrated with Hexamethyldisilazane (HMDS) and anhydrous ethanol, and then coated with a conductive layer prior to the measurement. For Micro-CT detection, the scaffold specimens were scanned from top to bottom in z-axis stack with 5 μm intervals and a peak voltage of 60 kV. The images detected by SEM and Micro CT were all managed with Image J software to obtain the interest dimensions of the tubular structure.

Statistical analysis In order to determine the statistical significance of experimental data for gel fraction and dimensions analysis, one-way analysis of variance (ANOVA), with a significance level $p < 0.05$ was performed (Minitab 16).

RESULTS

Defects of the printed VTES The experiments were carried out on the Biological 3D printing platform (home-built equipment, Shanghai University, China), as shown in Fig. S1A. The constant dripping of the core fluid at the head of the printed fiber could be clearly observed, as shown in Fig. S1B. This phenomenon could prove that the printed tubular architecture was unblocked.

For different formulations of sodium alginate and calcium chloride, the results showed different morphology. Three kinds of typical defects appeared. While the gel fraction was insufficient, the uncured sol would extend and occupy the designed space between adjacent fibers (Fig. 3A); at the same time, the uncured layer had a tendency to separate from the cured tubular fiber, especially at the turning corner, as shown in Fig. 3B; on the contrary, while the fiber was completely gelled, the surface of printed tubular fiber became