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Fast Contact of Solid–Liquid Interface Created High Strength Multi-Layered Cellulose Hydrogels with Controllable Size

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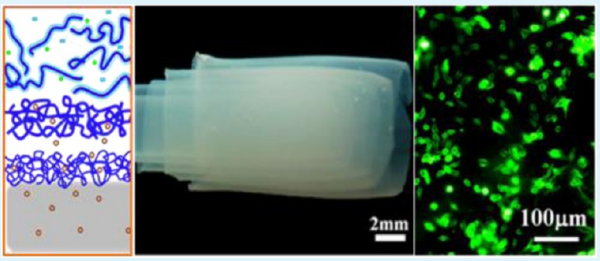
Supporting Information

ABSTRACT: Novel onion-like and multi-layered tubular cellulose hydrogels were constructed, for the first time, from the cellulose solution in a 7% NaOH/12% urea aqueous solvent by changing the shape of the gel cores. In our findings, the contacting of the cellulose solution with the surface of the agarose gel rod or sphere loaded with acetic acid led to the close chain packing to form immediately a gel layer, as a result of the destruction of the cellulose inclusion complex by acid through inducing the cellulose self-aggregation. Subsequently, multi-layered cellulose hydrogels were fabricated via a multi-step interrupted gelation process. The size, layer thickness and inter-layer space of the multi-layered hydrogels could be controlled by adjusting the cellulose concentrations, the gel core diameter and the contacting time of the solid–liquid interface. The multi-layered cellulose hydrogels displayed good architectural stability and solvent resistance. Moreover, the hydrogels exhibited high compressive strength and excellent biocompatibility. L929 cells could adhere and proliferate on the surface of the layers and in interior space, showing great potential as tissue engineering scaffolds and cell culture carrier. This work opens up a new avenue for the construction of the high strength multi-layered cellulose hydrogels formed from inner to outside via a fast contact of solid–liquid interface.



KEYWORDS: *multi-layered cellulose hydrogel, interfacial interaction, high compressive strength, biocompatibility, scaffold material*

1. INTRODUCTION

Recently, hydrogels have attracted much attention due to their fascinating properties such as moldability, high stretchability and stimuli response.[1−5] Furthermore, hydrogels with unique structures such as slide-ring and double network (DN) have become a hot topic, because special structures usually assure excellent properties such as high strength.[6,7] Multi-layered hydrogel, as a novel kind of hydrogels, exhibits complex internal structure with a large inter-layer space to store cells or drugs and controllable physical properties, which are very important in the biomedical fields.[8,9] Mimicking onion or blood vessels,[10] the multi-layered hydrogels can be used widely in biomedical fields such as in cell bioreactors,[11] micropatterning neural cell cultures,[12] tissue engineering[13] and drug delivery.[9,14] It is noted that polyelectrolyte biopolymers such as chitosan, alginate, sodium hyaluronate (HA) and carboxymethylcellulose (CMC) have been used to fabricate multi-layered hydrogels, which exhibit extraordinary advantages and characteristics.[9,10,13,15−17] Moreover, some synthetic polymer-based multi-layered hydrogels have also been prepared to show very fascinating applications.[18] More recently, it has been reported that the first gel membrane can be formed around a gel-core template when the gel-core, loaded with a crosslinker, is soaked in chitosan solutions through the crosslinking on the gel-core surface, so crosslinked multi-membrane chitosan hydrogels have been fabricated by repeating the soaking process.[15]

Renewable and environment friendly raw materials such as cellulose have attracted more and more attentions due to urgent demands for sustainable development all over the world. As a candidate of the promising biomaterial for cell culture and biomedical applications,[19,20] cellulose is a safe, nontoxic and biodegradable biopolymer with unique properties.[21,22] However, multi-layered cellulose hydrogels prepared directly from cellulose solution have never been reported, because cellulose is difficult to dissolve for further processing. In our laboratory, a novel NaOH/urea aqueous solution, with cooling, has been developed to dissolve cellulose and to fabricate the cellulose materials.[23−26] Compared with other organic solvents dissolving cellulose by heating method, the rapid dissolution of cellulose at low temperature in our aqueous solvents is attractive, safer and more environment friendly. Furthermore, a series of cellulose-based functional gels such as pH- and salt-responsive,[27] fluorescent hydrogels[28] and flexible cellulose/silica aerogels[29] have also been fabricated, showing smart behaviors and high strength. It is worth noting that a water-soluble inclusion complex (IC) associated with cellulose, NaOH, urea and water occurs in the NaOH/urea solvent system at low temperature, where the NaOH/urea shell formed through hydrogen bonds surrounds the cellulose chains, leading

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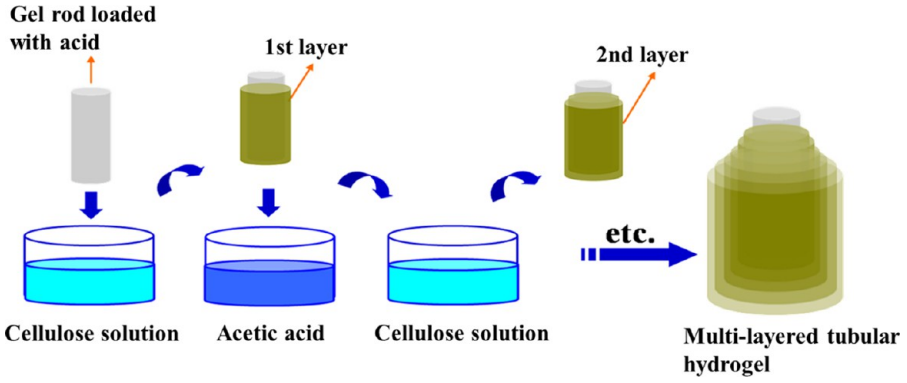
Scheme 1. Preparation Process of the Multi-Layered Cellulose Hydrogels



to the rapid dissolution.[23] Thus, once the IC is broken at elevated temperature or by adding acid, the −OH groups of cellulose will be exposed to induce the self-aggregation of cellulose to form rapidly physical hydrogels.[30] A worthwhile endeavor would be to find a novel and facile method to construct multi-layered cellulose hydrogels through the fast contact of the solid−liquid interface between the agarose gel core loaded with acid and the cellulose solution, which induced the cellulose aggregation due to the destruction of the cellulose IC by acid. The general approach to fabricate multi-membrane hydrogels is to prepare an entire hydrogel first, and then the first membrane is created by chain condensation and shrinkage through reaction for a relative long time, so the multi-membranes form from outside to inner via the multi-step process.[10] However, novel cellulose multi-layer formation occurs from inside to outside, which is fast because the breaking of cellulose IC is very rapid when contacting with acid. In the present work, a thermoresponsive biopolymer−agarose was used as the template core to control the architectures of cellulose hydrogels, because agarose can be gelled to form various required shapes and removed easily using simple heating and cooling method.[15] Cellulose hydrogels with multi-layered tubular and onion-like architectures were fabricated by changing the shape of the agarose cores. Meanwhile, the effects of the cellulose concentrations, the gel core diameter and contacting time of the solid−liquid interface on the layer thickness and inter-layer space were investigated to achieve the high strength multi-layered hydrogels with controllable architecture and size. The stiff chains of the intransigent cellulose can contribute good solvent resistance and high compressive strength for the multi-layered hydrogels. The cell adhesion and proliferation on the multi-layered hydrogels as well as the potential applications in biomaterials were also evaluated. The multi-layered cellulose hydrogels would be important for the application in the biomedical field. Thus, the fabrication of the multi-layered hydrogels by utilizing cellulose via the "green" process including NaOH/urea solvent and acetic acid would not only provide a facile method to create a novel kind of cellulose material but also broaden the application of cellulose in the biomedical field.

2. EXPERIMENTAL SECTION

2.1. Materials. The cellulose sample (cotton linter pulp) was supplied by Hubei Chemical Fiber Co. Ltd. (Xiangfan, China). Its viscosity-average molecular weight (M_η) was determined by using an Ubbelohde viscometer in a LiOH/urea aqueous solution at 25 ± 0.05 °C and calculated from the equation $[\eta] = 3.72 \times 10^{-2} M_W^{0.77}$ to be 10.0 × 10^4 g/mol.[31] 3,3′-Dioctadecyloxacarbocyanineperchlorate (DiO), for cell membrane staining, was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Agarose was purchased from Sinopharm Chemical reagent Co., Ltd. Poly-Llysine was bought from Sigma. NaOH, urea, acetic acid, cyclohexane, ethanol, acetone and dimethylacetamide (DMAc) were of analytical grade (Shanghai Chemical Reagent Co. Ltd., China) and used without further purification.

2.2. Preparation of the Multi-Layered Cellulose Gels. A 7 wt % NaOH and 12 wt % urea aqueous solution was pre-cooled to −12.8 °C, and then the desired amounts of cellulose sample were added immediately for dissolution. The cellulose was completely dissolved within 5 min with stirring to obtain a transparent solution, which was centrifuged to degas at 6000 rpm for 15 min. The gel rod (4.3 mm in diameter) was fabricated from a 2 wt % agarose solution at 90 °C in a plastic tubular mold after complete gelation at 5 °C in a refrigerator. The gel spheres with different sizes were prepared by dripping the agarose solution into cyclohexane with magnetic stirring. Two gel spheres of 2 and 3.9 mm in diameter were used here, referred to as small (S) and big spherical gel core (B), respectively.

The preparation process for the multi-layered tubular cellulose hydrogels is shown in Scheme 1. First, the agarose gel rod was immersed into the 10 wt % acetic acid for 1−10 min, rinsed with distilled water and then blotted with filter paper to remove the exterior acetic acid. Subsequently, the gel rod loaded with acetic acid was contacted with a cellulose solution on the solution−solid interface for the same time to prepare the first cellulose layer. The gel rod with the first layer was immersed again in the acetic acid, so the new gel rod loaded with acetic acid again could be used to form the second layer. The multi-layered cellulose hydrogels were generated by repeating this process, and the hydrogels fabricated from a gel rod with 3 (1) wt % cellulose concentration and 3 (10) min contacting time were coded as R3-3 (R1-10). Moreover, the hydrogels fabricated by using the big (small) gel sphere with 3 (1) wt % cellulose concentration and 3 min contacting time were coded as B3-3 (S1-3), respectively. The agarose gel cores can be removed easily by immersing into abundant water of above 90 °C to give the hollow multi-layered cellulose hydrogels.

2.3. Instruments and Characterization. The appearance and structure of the multi-layered cellulose hydrogels were observed by a biological microscope (EX20, Sunny, China). Scanning electron microscopy (SEM) observation was carried out with a Hitachi X-650 microscope (Mountain View, CA, Japan) and FESEM (SIRION TMP, FEI). The acceleration voltage for the FESEM observation was 10 or 12 kV. The cellulose hydrogels were frozen in a refrigerator or liquid nitrogen, freeze-dried and sputtered with platinum and then were observed and photographed. The compression strength of the tubular hydrogels was measured on a universal tensile tester (CMT 6503, Shenzhen SANS Test machine Co. Ltd., Shenzhen, China) according to ISO527-3-1995 (E) at a speed of 1 mm/min^{-1}. Because the strength data are related to the environmental temperature and humidity, these data were measured under the same conditions. The solvent resistance test was performed by immersing the B3-3 hydrogel in ethanol, DMAc, acetone and 10 wt % NaOH solution at 25 °C for 24 h, and then the hydrogel was taken out and photographed.

2.4. L929 Cell culture. The multi-layered tubular cellulose hydrogels were cut into cylinders with a height of about 0.3 cm, sterilized by autoclaving, soaked in the 0.1 mg/mL poly-Llysine for 0.5 h and then transferred to the bottom of 24-well plastic culture plates. A fresh Fibroblast cell (L929 cell) suspension (1 mL) was added to each triplicate sample, and the cellulose hydrogels were submerged totally with the suspension. After 3−4 h, the samples were supplemented with 1 mL of Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS). The L929 cells, with a cell density of 2×10^5 cells/cm^3, were co-cultured with cellulose hydrogels in an incubator for 2 days. The cellulose hydrogels with cultured L929 cells were then washed with PBS, fixed in 4 wt % paraformaldehyde, and post-fixed with 1 % OsO_4 for 24 h. After they were washed again with PBS for 3 times, they were frozen in a refrigerator and freeze-dried and then were coated with platinum for SEM observation. To observe and photograph L929 cells, the cell membranes were stained by using a 3,3′-dioctadecyloxacarbocy anineperchlorate (DiO) dye. The cellulose hydrogels with cultured L929 cells were immersed in a 10 μM DiO solution to be treated for 20 min at 37 °C, and then washed with PBS for 3 times. The cellulose hydrogels with cells treated by dyes were observed and imaged using a fluorescence microscope (Nikon ECLIPSETE 2000-U) at the corresponding excitation wavelength (Ex/Em = 484/501 nm). The experimental section for the cell viability by 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) assay is supplied in the Supporting Information.

3. RESULTS AND DISCUSSION

3.1. Tubular Multi-Layered Hydrogels. As shown in Scheme 1, from the cellulose solution dissolved in the 7 wt % NaOH and 12 wt % urea aqueous system with cooling, the multi-layered tubular hydrogels were fabricated successfully via a multi-step interrupted gelation process. Figure 1a,b shows the photographs of the multi-layered tubular cellulose hydrogels from 1.5 and 3 wt % cellulose solutions, respectively. Clearly, the cellulose layer was smooth, indicating that the formation of hydrogels was a uniform physical process. R1.5-10 and R3-10 displayed tubular architectures with different layer thicknesses, depending on the cellulose concentration. As it was expected, the tubular multi-layered cellulose hydrogels with inter-layer space could be constructed easily. A schematic presentation for the formation of the multi-layered cellulose hydrogels is proposed in Scheme 2. When the cellulose IC was destroyed with acid through the fast contact of the solid−liquid interface between the gel rod with acid and the cellulose solution, the strong inter-chain interactions of the exposed cellulose chains led the rapid self-aggregation of cellulose, so the first cellulose layer formed immediately along the gel core. Subsequently, the first layer was cured by immersing in acetic acid solution again, and the cellulose layer could shrink due to the further aggregation. Furthermore, the resultant cellulose/agarose gel loaded with acetic acid served as a new "gel core" to regenerate the next cellulose hydrogel layer. The formation of inter-membrane spaces could be promoted by prolonging the curing, which could give sufficient time for the rearrangement and self-aggregation of the cellulose chains, resulting in an increase of the gel density, namely shrinkage of the gel volume. Thus, the inter-layer space formed with a progress of the curing process. In view of the presentation of Scheme 2, the diameter of the cellulose tubes increased from interior to outside in the hydrogel with the repeating process, as shown in Figure 1a,b. Thus, the diameter and layer thickness of the cellulose hydrogel tubes could be controlled by changing the cellulose concentration and the repeating process. SEM images of the multi-layered hydrogels (Figure S1, Supporting Information) indicated that the original appearance of the multi-layered

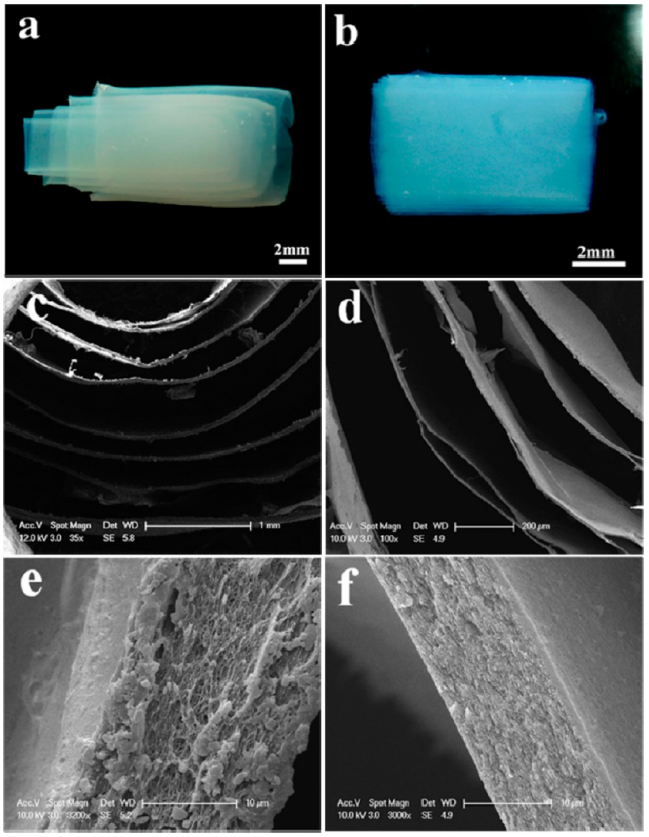
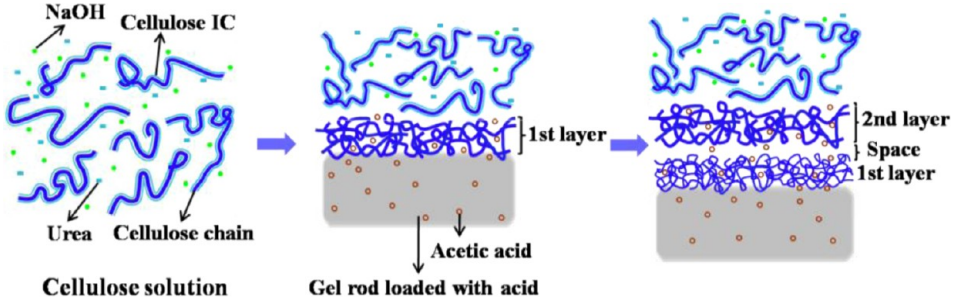


Figure 1. Photographs of the multi-layered tubular cellulose hydrogels (a,b) and the corresponding SEM images (c,e and d,f) for the cross-section of the freeze-dried gels fabricated from different cellulose concentrations of 3 wt % for R3-10 (a,c,e) and 1.5 wt % for R1.5-10 (b,d,f), respectively.

architecture was well maintained during the drying process. It was noted that their homogenous porous structure with the pore size of about 2 μm and wide inner space of about 70 μm could provide sufficient space for normal cell adhesion (the size is about 20 μm) and transportation of nutriments.[32] In our findings, the stiff and hydrophilic cellulose chains contributed to support the thin wall of pores, leading to the regular macroporous structure. The SEM images of the freeze-dried cellulose hydrogels frozen at −20 °C are shown in Figure 1c−f. It was further confirmed that the cellulose hydrogels had the multi-layered architecture and could be used as the scaffolds. With an increase of the cellulose concentration, the layer thickness increased. Interestingly, the distance between the layers of the multi-layered hydrogels was about 100−200 μm, which is suitable for cell culture and biomedical applications, so it was important in the biomaterial field.[33]

3.2. Onion-like Hydrogels. The onion-like cellulose hydrogels were constructed by using an agarose gel sphere. Moreover, the effects of the cellulose concentration, the amount of loaded acetic acid and the soaking time on the layer thickness were investigated here. Figure 2 shows the photographs of the multi-layered cellulose hydrogels from different contacting and curing time. The average layer thickness value of ten measurements for a (c) was about 158 (206) μm, and it decreased to 103 (128) μm after curing for 10 min in acetic acid (b, d). The results indicated that the layers fabricated by contacting for 1 min were compactly connected without an inter-layer space. However, the layers thickness

Scheme 2. Schematic Model to Describe the Formation of the Multi-Layered Cellulose Hydrogel via Fast Solution−Solid Interfacial Interaction



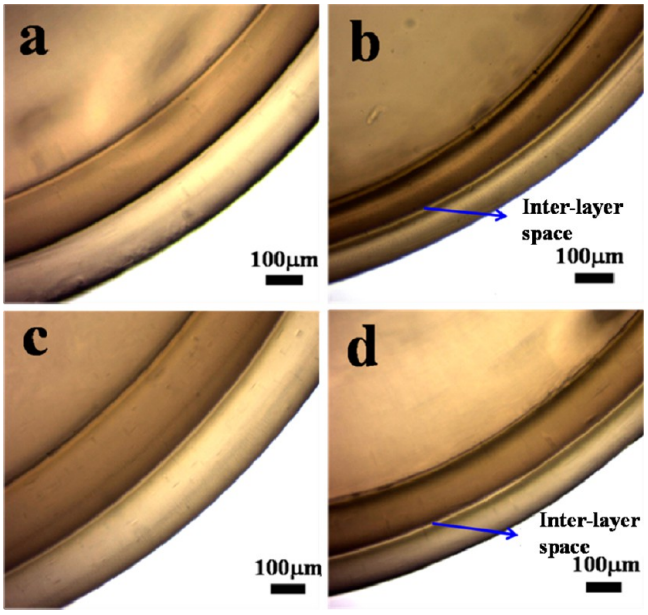


Figure 2. Photographs of the multi-layered cellulose hydrogels from 3 wt % cellulose concentration at different contacting times: 1 min (a) and 3 min (c); b, d represent a, c after being further cured for 10 min, respectively.

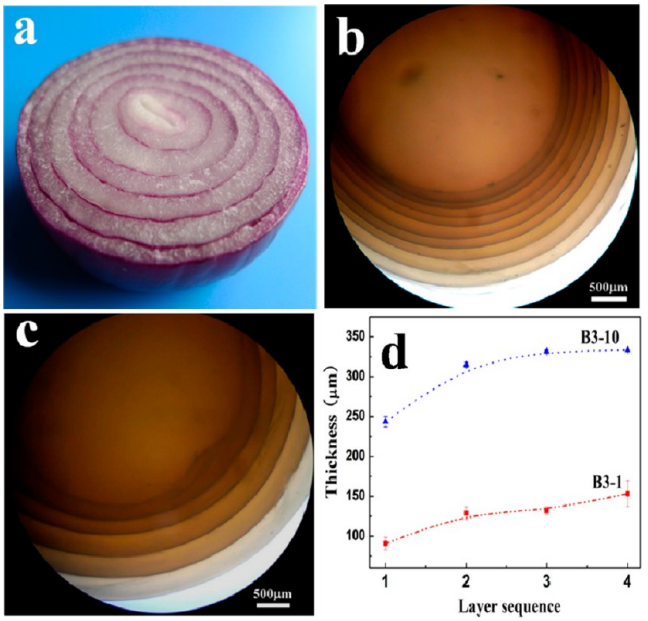


Figure 3. Photographs of an onion (a) and the multi-layered cellulose hydrogels of B 3-1 (b), B3-10 (c) fabricated from the big spherical gel core with 3 wt % cellulose concentration treated for 1 min and 10 min, respectively, and the corresponding layer thickness of the cellulose hydrogel as a function of the layer sequence (d).

decreased evenly by further curing for 10 min, namely shrinkage appeared, resulting in an inter-layer space. Interestingly, a clear multi-layered cellulose hydrogel contacted for 3 min appeared after being cured for 10 min. This could be explained that a sufficient curing time was essential for the perfect rearrangement and self-aggregation of the cellulose chains. In our findings, the prolonged curing time induced the relatively close chain packing, resulting in the corresponding formation of an inter-layer space. Therefore, the additional curing time was beneficial for the distinct inter-layer space formation of the cellulose layer, as a result of the contraction of the hydrogel layers. As mentioned above, the construction of the multi-layered cellulose hydrogels was performed from inner to outside. It was not hard to imagine that this kind of layer-by-layer assembly could be easier to control the layer number and inter-layer space. The controllable architecture and inter layer space are important for the incorporation of biological components.[34]

3.3. Controllable Architecture and Size. Onion-like multi-layered cellulose gels were used to study the influence of the controllable factors on the layer architecture and thickness. Figure 3a−c shows the photographs of a natural onion and spherical multi-layered cellulose hydrogels from different contacting times. Clearly, the multi-layered cellulose hydrogels were similar to the onion architecture. The layers in the hydrogels were very compact and regular, and the layer thickness increased with an increase of contacting time of the solid−liquid interface (b,c) or with the enhancement of the sequence number (Figure 3d). As mentioned above, the diameter of agarose/cellulose gel core was larger than that of agarose gel core, and the "core" diameter increased with the repeating process (Scheme 2). So, the larger the sequence number, the larger the gel core diameter was, because the larger gel core could load more acetic acid to break more cellulose ICs to induce self-aggregation of more cellulose, resulting in the thicker and larger layers. Figure 4 shows the photographs of the multi-layered cellulose hydrogels constructed from the different core diameters and cellulose concentrations, as well as the plots of the corresponding layer thickness as a function of the layer sequence. The results indicated that both the larger diameter of the gel core and a higher cellulose concentration could lead to an increase of the layer thickness. The conclusion was in good agreement with that of the tubular hydrogels. Additionally, the layer thickness also increased slightly with the increment of the

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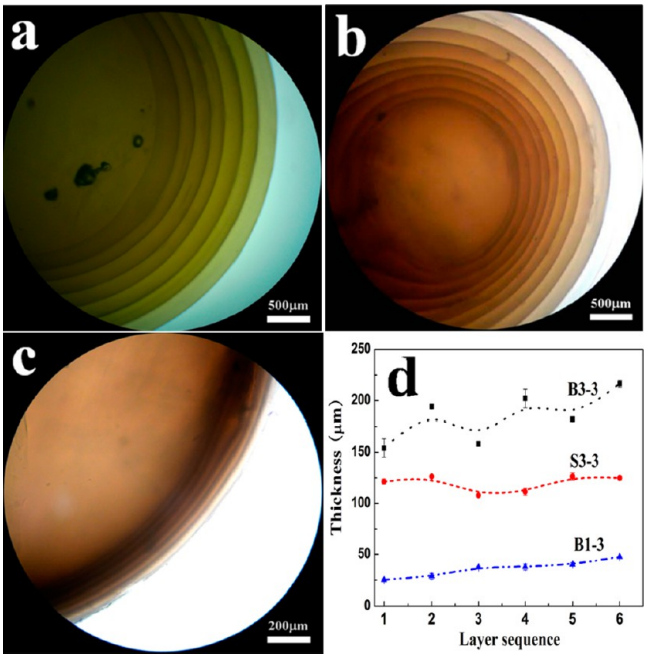

Figure 4. Photographs of the multi-layered cellulose hydrogels of B3-3 (a), S3-3 (b) and B1-3 (c) fabricated from the big, small and big gel cores with 3 wt %, 3 wt % and 1 wt % cellulose concentrations respectively, and the layer thickness of the cellulose hydrogel as a function of the layer sequence (d).

sequence number on the whole. In view of above results, the cellulose layer thickness could be controllable in a wide range from 36 to 305 μm by adjusting the cellulose concentration, gel core diameter and the contacting time. The suitable range of the concentration and contacting time for the multi-layered cellulose hydrogels were much wider than that of the reported results. Moreover, the formation cycle for a cellulose layer (about 2 min at fastest) was much faster than that of a chitosan layer (8 min) reported by Ladet et al.,[10] in which acid−base neutralization induced chitosan chain condensation. This could be explained by the rapid dissolution of cellulose in the NaOH/urea solvent through a fast dynamic self-assembly between solvent and macromolecules,[23] thus cellulose chains self-aggregated instantly to form the gels when the NaOH/urea shell was broken by acid. It was noted that the large inter-layer space separated by cellulose layers could be used as a cell bioreactor, which would be beneficial for the cell growth and mass exchange.[35] The controllable layer thickness of the multi-layered hydrogels was very important for their applications, because varied sizes were required in different fields such as blood vessels and cortical neuron cell with different layer thicknesses.[12] Therefore, our work has opened up a new avenue for the construction of the multi-layered cellulose hydrogels with controllable architecture via a facile and rapid pathway.

3.4. Physical Properties and Biocompatibilities. Usually, the physical hydrogels fabricated from natural polymers, such as chitiosan, alginate, sodium hyaluronate and carboxymethyl cellulose, are poor in solvent resistance against water and organic solvents. To demonstrate the chemical stability of the cellulose hydrogels, B3-3 was immersed in four kinds of solvents (ethanol, acetone, DMAc and 10 wt % NaOH aqueous solution) at 25 °C for 24 h to measure the swollen or dissolution behaviors. Figure 5 shows the photographs of the B3-3 before and after being immersed in different solvents. Clearly, the appearance of the multi-layered architecture hardly changed, and no layer was broken. The good solvent resistance was attributed to the intransigent character of cellulose molecules. The good solvent resistance of the multi-layered cellulose hydrogels would be crucial for wide applications in diverse solvents.

The good layer elasticities and strength are essential for the application in the mechanical responses of the multi-layered hydrogels at external pressure.[36] The compressive stress−strain curves of the multi-layered cellulose hydrogels are shown in Figure 6. A multi-layered tubular cellulose hydrogel (the inset of the Figure 6) was adopted for the testing. All of the multi-layered cellulose hydrogels fabricated by non-covalent bonding exhibited compressive strength higher than 0.3 MPa, whereas strain was more than 55%. The compressive strength was much higher than that of both physical and chemical crosslinked cellulose hydrogels at the same concentration, such as about 30 kPa for the chemical-crosslinked cellulose hydrogels.[37] Therefore, the multi-layered cellulose hydrogels exhibited high compressive strength, showing the robust layers. Furthermore,

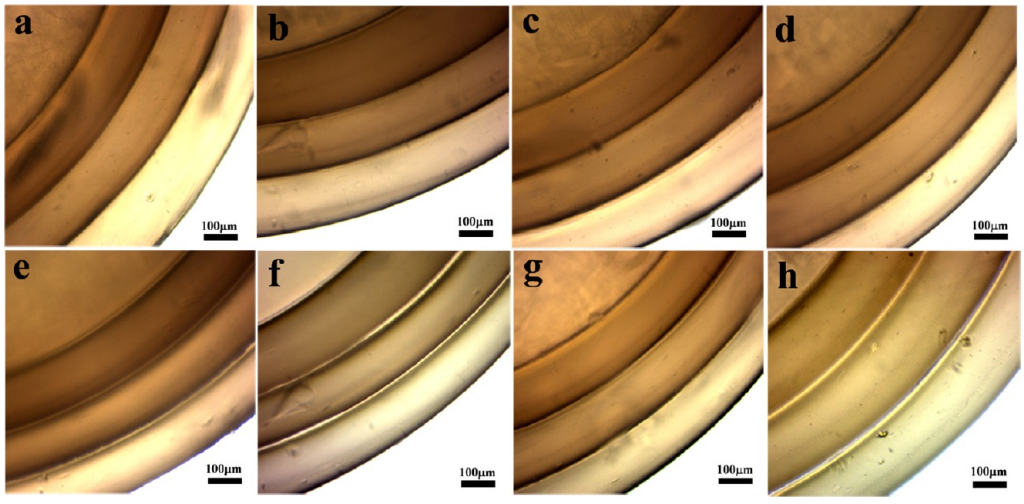

Figure 5. Photographs of the B3-3 before and after being immersed, respectively, in ethanol (a,e), DMAc (b,f), acetone (c,g) and 10 wt % NaOH solution (d,h) for 24 h.

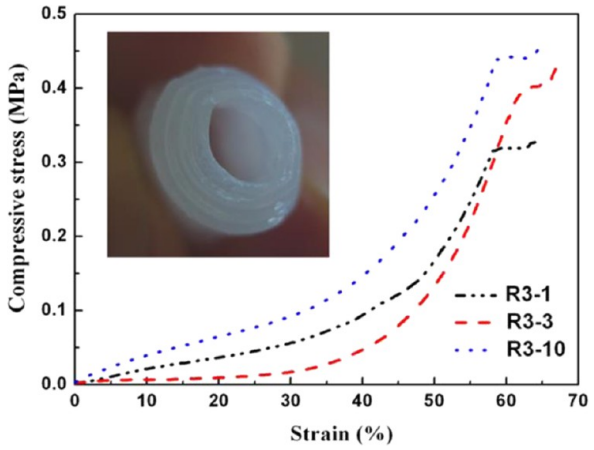

Figure 6. Compressive stress−strain curves of the R3-1, R3-3 and R3-10 hydrogels and the photograph of the R3-3 (inset).

the compressive strength increased with the increment of contacting time, as a result of the increase of layer thickness and the aggregate density.

Sufficient space in the scaffolds is a key factor for cell culture and cell proliferation.[33] To evaluate the potential applications of the multi-layered cellulose hydrogels as cell reactors or biomaterials, L929 cells were used as seed cells to test their adhesion, migration and distribution. For the morphology of the control (Figure S2, Supporting Information), the L929 cells exhibited a shuttle-shape and some dead cells appeared. After L929 cells were seeded between the layers for 2 days, the cells were found to adhere, spread and proliferate on the multi-layered cellulose hydrogels, as shown in Figure 7. In our findings, the L929 cells appeared on the layer surface, and uniformly distributed in the inter-layer space. It was noted that the multi-layered cellulose hydrogels provided not only sufficient pores for the cells to be immobilized and to migrate but also offered sufficient space and large area for the cells to proliferate and exchange nutrients and metabolite wastes. These are vital for cell culture and cell growth.[38] Compared with the control, the cell density for the multi-layered cellulose hydrogels was relatively low because the surface area for cell to adhere was much larger, and there were relatively fewer dead cells, so the multi-layered hydrogels were suitable for the L929 cell culture. Figure 8 shows the fluorescent images of the cell

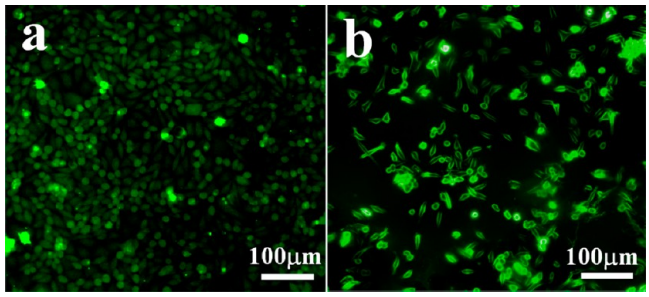

Figure 8. Fluorescent images of cells cultured on the control (a) and cellulose hydrogels (b) for 2 days (stained with DiO).

culture after 2 days. A single layer from the multi-layered cellulose hydrogels was used for observation, so the morphology of L929 cells on the layer surface can be easily observed, which served as the supplementary to the three dimensional observation from the vertical direction by FESEM (Figure 7). Most of the cells stained by DiO on the cellulose layer exhibited normal shuttle-shape and less cells dead compared with the control, confirming that the cellulose hydrogels were cytocompatible to the L929 cells (Figure 8b) and the space was important for cell culture. This indicated that the cellulose hydrogels were a good candidate for the cell culture and proliferation, as mentioned in references.[39,40] In view of the above results, the robust multi-layered cellulose hydrogels exhibited excellent biocompatibility to the L929 cells, and could be used as a cell reactor for cell culture at large scale or as scaffolds for tissue engineering field. The cell cytotoxicity studies were also performed to evaluate their potential biomedical applications. The MTT assay results of the gels (Figure S3, Supporting Information) indicated that the cell viability values on all gels were higher than that for the control, indicating non-cytotoxicity and good biocompatibility to L929 cells. This was as a result of the nontoxicity of cellulose itself, which exists extensively in plants and some bacteria. Therefore, the multi-layered cellulose gels exhibited good capacity for cell

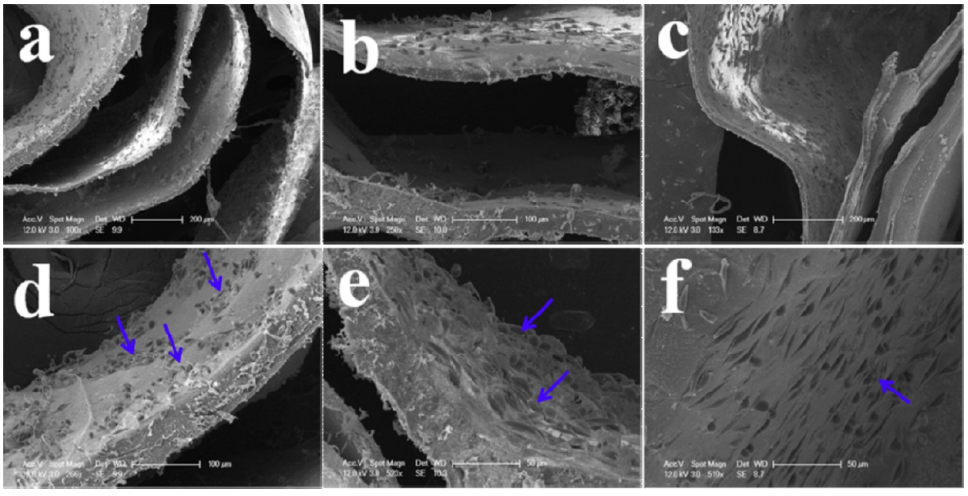

Figure 7. Morphologies of the L929 cells cultured for 2 days on the layer surface of the multi-layered cellulose hydrogels: R3-3(a,d); R2-3(b,e); R1.5-3(c,f). The arrows showed the L929 cells.

proliferation with potential applications as cell reactor or tissue scaffold.

4. CONCLUSIONS

Novel onion-like and multi-layered tubular cellulose hydrogels were constructed successfully through a fast contact of the solid−liquid interface between the gel rod or sphere loaded with acid and the cellulose solution. Due to the instant destruction of the cellulose IC by contact with acid on the surface of the gel core, the cellulose hydrogel layer could be constructed rapidly along the gel surface through the quick self-aggregation between exposed cellulose chains. The layer thickness and inter-layer space could be controlled by adjusting the cellulose concentrations, the gel core diameter and the contacting time of the solid−liquid interface. The multi-layered cellulose hydrogels exhibited high compressive strength, as a result of the relatively close cellulose chain packing. The hydrogels had good architectural stability and solvent resistance against ethanol, acetone, DMAc and NaOH aqueous solution. The L929 cells could adhere and proliferate on the surface of the layers and in the inter-layer space, showing non-cytotoxicity and good biocompatibility. The controllable architecture and layer size of the multi-layered cellulose hydrogels are important in the application as biomedical materials.

■ ASSOCIATED CONTENT

Supporting Information

SEM image of the multi-layered hydrogel from the freezing dried process; the control for Figure 7; MTT assay experimental process and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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