Shear viscoelasticity of electrospinning PCL nanofibers reinforced alginate hydrogels

Lu Pang¹, Peixin Sun¹, Xufeng Dong¹*, Tao Tang³, Yi Chen¹, Qiang Liu¹ and Min Qi¹*

¹ School of Materials Science and Engineering, Dalian University of Technology, Dalian 116024, People’s Republic of China
² Departments of Neurosurgery, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang 110042, People’s Republic of China
* Authors to whom any correspondence should be addressed.

E-mail: dongxf@dlut.edu.cn and minqi@dlut.edu.cn

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Abstract

Articular cartilage has limited self-repair capacity due to the lack of vascularization, innervation and lymphatic networks. Biomimetic scaffolds with features of the extracellular matrix (ECM) of cartilage are advantageous to repair the injured cartilage tissue, but it remains a challenge to regulate its shear viscoelasticity to meet the needs of applications as articular cartilages. Fiber reinforced hydrogel is of great significance for their clinical application as cartilage tissue engineering scaffolds, especially for repairing the fibrocartilage tissue like meniscus or temporomandibular joint disc. In order to promote the shear viscoelasticity of alginate hydrogels, which was seldom studied, electrospinning PCL nanofibers layers were added into the alginate hydrogels to prepare PCL nanofibers reinforced alginate hydrogel composites (PNRAHCs). Compared with neat alginate hydrogel scaffolds, the PNRAHCs presented coral-like structure and spider web-like structure, and some PCL nanofibers form reinforced fiber bundles. Those special structures make the PNRAHCs have higher porosity, higher shear storage modulus and higher shear loss modulus than the neat alginate hydrogels, indicating better shear mechanical properties. They have the potential to be applied as the scaffolds to repair fibrocartilage tissues.

1. Introduction

Articular cartilage damage is a common orthopedic disease which has limited self-repair capacity due to the lack of vascularization, innervation and lymphatic networks [1, 2]. During the past decades, clinical progress has been made in repairing damaged cartilage. The methods that have been proposed include medical treatment, arthroplasty, discectomy and synthetic or alloplastic cartilage replacement [3]. Recently, tissue engineering has demonstrated great potential for clinical application owing to the successful reconstruction of morphology, structure and function of damaged cartilage [4–6]. Hydrogels, which have high water content and porous framework that is similar to the natural extracellular matrix (ECM), have been successfully used as cartilage tissue engineering scaffolds [7–9]. For instance, Park et al modified alginate hydrogels with low molecular weight hyalurionate [10]. The composite gels had a defined mechanical stiffness and promoted chondrogenic differentiation compared with unmodified alginate hydrogels. Fernandez et al developed gene-activated alginate hydrogels capable of supporting nanohydroxyapatite (nHA)-mediated nonviral gene transfer to control the phenotype of mesenchymal stem cells (MSCs) for either cartilage or endochondral bone tissue engineering [11]. Liao developed an injectable three-dimensional (3D) alginate hydrogel loaded with biodegradable porous poly (ε-caprolactone)–b-poly-(ethylene glycol)–b-poly(ε-caprolactone) microspheres (MPs/Alg) as the calcium gluconate container to cross-link alginate [12].

Nevertheless, the treatment of fibrocartilaginous tissue like meniscus and temporomandibular joint disc is still a challenge [13]. Compared with common hyaline cartilage, fibrocartilage is stiffer since the fibrochondrocytes produce higher levels of collagen type I [14]. Furthermore, fibrocartilage tissues usually have complex structures like random collagen bundles or hierarchical structure, which makes the therapy become
more difficult [15]. To improve the stiffness of hydrogels, reinforced hydrogels have been developed and presented great prospect in fibrocartilage treatment [16–22]. Fiber strands, fiber layers and woven structures have been used to reinforce hydrogels. For example, Visser and co-workers reinforced soft gelatin methacrylate (GelMA) hydrogels with highly organized, high-porosity PCL microfibers [23]. The stiffness of the composite hydrogels increased synergistically (up to 54-fold) compared with the pure GelMA hydrogels. Kim et al prepared a PCL scaffold with a hierarchical structure consisting of alternating layers of perpendicular strands and thin nanofiber webs [24]. The hybrid scaffold showed controlled mechanical properties. Liao et al fabricated a three-dimensional PCL woven fiber scaffold with an inter-penetrating alginate/polyacrylamide hydrogel network [25]. The fiber-reinforced composite structure provided the scaffolds with higher load-bearing capacity and improved its Young’s modulus and dynamic moduli.

Most of the above studies mainly focused on the improvement of compression or tension properties of hydrogel scaffolds [26, 27]. However, joint loading is a complex combination of compressive, tensile and shear [28–30]. Articular cartilages are subjected to external shear forces up to several times body weight for millions of cycles over a lifetime [31]. Therefore, the dynamic shear viscoelasticity is a crucial mechanical property which must be considered. For most of hydrogels, their shear viscoelasticity cannot meet the needs of applications as articular cartilages. To mimic the biomechanical properties of articular cartilages, the shear viscoelasticity of hydrogel scaffolds should be enhanced, but to the best of our knowledge, it has been seldom studied.

Sodium alginate is a natural polysaccharide with good biocompatibilities and biodegradabilities, but the low shear stiffness has limited its applications in cartilage scaffolds [32–34]. Deepthi’s study indicated that the multilayered structures with hydrogel matrix and reinforced nanofibers have great potential in increasing shear modulus of chitosan-collagen hydrogels [35]. Furthermore, the layered structures have advantages in improving cell seeding and promoting biomineralization [36, 37]. Inspired by those studies, multilayer PCL nanofibers, which were synthetic biodegradable polymers with tunable mechanical properties, were used to reinforce the shear properties of the alginate hydrogels in this study. The PCL nanofibers reinforced alginate hydrogel composites (PNRAHCs) would be potentially used as fibrocartilage tissue engineering scaffolds. Electrospinning is an outstanding method to generate no-woven fiber structures or continuous polymer fibers with morphology similar to extracellular matrix (ECM) [38–40], and the electrospinning fibers have well-controlled diameters, high porosity and high surface area to volume ratio [41, 42]. Considering those merits, PCL nanofibers were prepared by the electrospinning method in this study. The shear viscoelasticity, specifically the dynamic shear moduli of the electrospinning PCL nanofibers reinforced alginate hydrogel composites were tested and compared with that of the alginate hydrogels.

2. Experimental

2.1. Materials

Sodium alginate was purchased from Tianjin Damao chemical reagents company. Polycaprolactone (MW = 80,000) was purchased from Shenzhen Guanghua Weiye Industrial Co., Ltd. The organic solvents dichloromethane (DCM) and N,N-dimethylformamide (DMF) were purchased from Tianjin Fuyu Fine Chemical Co., Ltd. The hydrogel activator 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC), crosslinker adipic acid dihydrazide (AAD) and the buffer 2-morpholinoethanesulfonic acid (MES) monohydrate were bought from Aladdin Biochemical Technology Co., Ltd. The ethanol and sodium chloride were purchased from Tianjin Damao chemical reagents company. All reagents were used without further purification. Deionized water used throughout the experiments was prepared by the Ultra-pure water purification system.

2.2. Synthesis of hydrogel, nanofiber and multilayered composite hydrogel

The sodium alginate was dissolved in a MES buffer (0.1 M MES and 0.5 M NaCl) to obtain a 15% (w/v) solution, then stirred for 5 h at room temperature to get a homogenous solution. Gelation was proceeded by adding an aqueous solution of EDC (molar ratio of EDC to sodium alginate was 1:2) and AAD (5 mM in the resulting solution) into the alginate solution with vigorous stirring.

PCL solution (12 wt% concentration) was prepared by dissolving the pre-weighed amount of PCL beads in DCM-DMF (7:3) solution and stirring for 12 h at ambient temperature, which was standing for 1 h just before the electrospinning process. The solution of PCL was loaded into a 5 ml plastic syringe fixed onto a syringe pump and squeezing out through a blunt 18 G needle (inner diameter 0.84 mm) at a flow rate of 0.8 ml h⁻¹ and a high DC voltage of 20 kV. Electrospinning was carried out at room condition (temperature = 25 °C, and humidity = 40%). The vertical distance between the tip of the needle and the collector was set at 15 cm. During the electrospinning, the nozzle was fixed vertically, and the fibers were collected on a tablet collector.

The PNRAHCs were prepared by electrospinning PCL nanofibers into alginate matrix layer by layer. The schematic of the preparation process is shown in figure 1. The bottom hydrogel layer was achieved by pouring
1 ml of sodium alginate solution into an aluminum foil–covered petri dish \((\varphi = 10 \text{ cm})\). The reinforced fibers were then added by electrospinning for 1 min. This routine was repeated 10 times, with a nanofiber layer on top. Gelation was achieved by injecting crosslinker solution into the composite hydrogels via a syringe. Then, the PNRAHCs were frozen at \(-20 ^\circ \text{C}\) overnight and then lyophilized 48 h for feature using.

2.3. Morphology and structural characterization

Morphology of PCL nanofibers, alginate hydrogels and PNRAHCs was characterized by scanning electron microscopy (SEM, SUPARR 55, Zeiss, Germany). Samples of cross section (top layer of PNRAHCs) and longitudinal section were sliced into \(10 \times 10 \times 2 \text{ mm}^3\), and sputter-coated with an ultrathin layer of aurum. The spectroscopy characterization was performed by Infrared Spectroscopy and Fourier Transform (FT-IR, iN10, Thermo Fisher) using a KBr pellet with 40 scans in the range of 4000–400 cm\(^{-1}\). The specific surface area and pore size distribution were measured by using an Automatic physical adsorption instrument measurement (MicroActive ASAP2020) at 77.3 K.

The porosity was examined with liquid displacement method using the pycnometer and calculated using equation (1) \([43]\)

\[
\varepsilon = \frac{(W_2 - W_1 - W_3)}{(W_1 - W_3)}
\]

where \(W_1\) is the weight of dry sample. \(W_1\) and \(W_2\) are the weights of samples and ethanol-filled pycnometer before and after vacuum pumping, respectively. \(W_3\) is the weight of pycnometer without samples after vacuum pumping. All experiments were repeated three times.

Swelling ratios of PCL nanofibers, alginate hydrogels and PNRAHCs were analyzed by comparing the dry mass of the lyophilized samples (\(~0.1\) g) with the mass of those measured at \(37 ^\circ \text{C}\) in deionized water (50 ml) after 48 h incubation. The swelling ratio was calculated using equation (2)

\[
\text{Swelling Ratio} = \frac{(W_{\text{wet}} - W_{\text{dry}})}{W_{\text{dry}}}
\]

where \(W_{\text{wet}}\) and \(W_{\text{dry}}\) are the weights of the fully swollen samples in aqueous solutions and in the dry state, respectively. All swelling experiments were repeated three times and the mean of the data were obtained for comparison.

2.4. Mechanical properties tests

Dynamic viscoelasticity of the fresh prepared alginate hydrogels and PNRAHCs was tested with a Physica MCR301 rheometer (Anton Paar) equipped with a PP20-E parallel plates system, which can apply dynamic shear force on soft materials and liquids. The testing gap and the diameter of the parallel plate was 1 mm and 20 mm, respectively. To examine the linear viscoelastic region of the samples, an amplitude sweep was taken in which the strain was logarithmically applied from 0.01% to 20% at a constant angular frequency of 5 rad s\(^{-1}\), which simulate frequency of normal physiological needs [29]. Then a frequency sweep was taken at a constant strain of 0.1%, under which the materials are in their linear viscoelasticity state, and logarithmically increased angular frequency from 1 rad s\(^{-1}\) to 100 rad s\(^{-1}\). During the process, the shear storage modulus and loss modulus of the samples were obtained. All the measurements were conducted at body temperature (37 °C). All the measurements were conducted three times. The alginate hydrogels and PNRAHCs didn’t collapse and maintained their structures after three times of tests. The values were expressed as mean values \(\pm\) standard deviations.
3. Results and discussion

Figure 2 shows the morphology of the alginate hydrogels and the neat PCL nanofibers. The alginate hydrogels are transparent and porous with large pores with size up to several hundred micrometers (figures 2(a), (b)). PCL nanofibers look like paper and the mean diameter of PCL nanofibers is around 600 nm (figures 2(c), (d)). The PNRAHC is translucent with PCL nanofibers in alginate hydrogel matrix. The microscopic level morphology of PNRAHCs is displayed in figure 3(a). On the cross section, the PNRAHCs have rough surface with tangled nanofibers fulfilled the alginate hydrogels pores (figure 3(b)), which increases the specific surface area. On the longitudinal section, PNRAHCs show porous and multi-layered structure (figure 3(c)). The PCL nanofibers connect with the alginate hydrogels, which would maintain the structure stability. By amplifying, detailed
microstructures can be observed, as shown in figures 3(d)–(f). The most common microstructure is ‘coral reef’-like structure (figure 3(d)). It is a loose structure with a staggered PCL nanofibers skeleton adhering some sodium alginate. With the sodium alginate wrapped outside, the ‘coral reef’-like structure may exhibit higher biocompatibility than neat PCL nanofibers. ‘Spider web’-like structure is another microstructure that can be found, as shown in figure 3(e). Electrospinning PCL nanofibers are woven into a thin web, and small amount of sodium alginate are suspended on nanofibers or gathered at the intersection of multiple nanofibers. With the thin PCL nanofiber webs fulfilled the pores of alginate hydrogel matrix, the ‘spider web’-like structure shows higher porosity than alginate hydrogels. Besides, there are some fiber bundles are wrapped by sodium alginate (figure 3(f)). Those fiber bundles appear in the gap between two layers, connecting the hydrogel matrix pore walls, and further reinforce the alginate hydrogels and increase the overall specific surface area.

The FT-IR spectra of PCL nanofibers, alginate hydrogels and PNRAHCs are shown in figure 4. The neat PCL nanofibers show absorption peaks at 2945 cm\(^{-1}\) of CH\(_2\) stretching, 1726 cm\(^{-1}\) of carbonyl stretching and 1240 cm\(^{-1}\) of C–O–C stretching [44]. Alginate hydrogels reveal characteristic peaks at 3450 cm\(^{-1}\) and 1630 cm\(^{-1}\) of O–H stretching and bending. By comparison with alginate hydrogels, O–H stretching and bending peaks in PNRAHCs shift to 3350 cm\(^{-1}\) and 1580 cm\(^{-1}\), respectively, which indicates hydrogen bonds are generated between the carboxyl groups and methylene groups of PCL nanofibers and the hydroxyl groups of alginate hydrogels [45].

The porosity of PCL nanofibers, alginate hydrogels and PNRAHCs are shown in figure 5(a). All samples exhibit high porosity about 90%. Among them, the PNRAHCs have the highest porosity up to 95%, which can

![Figure 4. FT-IR patterns of three samples: (a) PCL nanofibers, (b) alginate hydrogels, (c) PNRAHCs.](image)

![Figure 5. Porosity (a) and Swelling ratio (b) of PCL nanofibers, alginate hydrogels and PNRAHCs.](image)
be attributed to the extensive ‘coral reef’-like structure and ‘spider web’-like structure. In addition, Table 1 summarizes the surface area and adsorption average pore diameter of PCL nanofibers, alginate hydrogels and PNRAHCs. The specific surface area of PNRAHCs was 4.4491 m²g⁻¹, larger than the neat alginate of 3.4745 m²g⁻¹, and PCL nanofibers of 3.3504 m²g⁻¹. SEM comparison shows the specific ‘coral reef’-like structure and ‘spider web’-like structure, which may be the reason for the large BET surface area of PNRAHCs. Figure 5(b) shows the swelling ratio of PCL nanofibers, alginate hydrogels and PNRAHCs. Among the three kinds of materials, PCL nanofibers present the lowest swelling ratio (3.66), while the alginate hydrogels present the highest swelling ratio (11.45). The swelling ratio of PNRAHCs is 8.75, which is lower than that of the neat alginate hydrogels. The possible reason is that the ‘spider web’-like structure and fiber bundles caused by adding PCL nanofibers make the alginate hydrogels become more rigid, and so the water absorption ability declines.

Figure 6 shows the relationship between the shear viscoelasticity and the loading conditions. Figure 6(a) shows the dependence of the storage modulus ($G'$) and the loss modulus ($G''$) on strain amplitude at a fixed frequency of 5 rad s⁻¹. As the strain amplitude increases, the storage modulus of the alginate hydrogels and PNRAHCs keeps nearly unchanged at low strain amplitude level and then decreases when the strain amplitude exceeds a critical value. Compared with neat alginate hydrogels, the PNRAHCs exhibit higher storage modulus and loss modulus. The promoted storage modulus can be attributed to the reinforced effect by adding PCL nanofibers. The formation of coral reef-like structure, spider web-like structure and fiber bundles make the alginate hydrogels become stiffer. On the other hand, due to the interaction between the alginate hydrogels and PCL nanofibers, the energy dissipated during the loading process also increases, which make the PNRAHCs have higher loss modulus. Figure 6(b) shows the relationship between $G'$ and $G''$ of alginate hydrogels and PNRAHCs and the frequency at a fixed strain amplitude of 0.1%, under which the two samples are in their linear viscoelastic states. The storage modulus of the alginate hydrogels and PNRAHCs is constantly greater than their loss modulus, which is the evidence of typical gel networks. The frequency stable region of both hydrogels is from 2 rad s⁻¹ to 40 rad s⁻¹, which covers normal physiological needs [29]. Moreover, the PNRAHCs have a higher $G'$ and $G''$ than the alginate hydrogels, which is caused by adding electrospinning PCL nanofibers. The robust PCL nanofibers act as reinforced phase. The ‘spider web’-like structure and fiber bundles microstructure found in PNRAHCs have connected the hydrogel matrix and strengthened the composite hydrogels. Since adding the PCL nanofibers, the PNRAHCs network is harder to deform under the shearing environment, showing a higher storage modulus. The interaction of alginate hydrogels and PCL nanofibers forms a tangled structure and causes

Table 1. BET surface area and adsorption average pore diameter of PCL nanofibers, alginate hydrogels and PNRAHCs.

| Sample          | BET surface area (m² g⁻¹) | Adsorption average pore diameter (nm) |
|-----------------|---------------------------|--------------------------------------|
| PCL nanofibers  | 3.3504                    | 12.0075                              |
| Alginate hydrogels | 3.4745                  | 17.6674                              |
| PNRAHCs         | 4.4491                    | 8.0599                               |

Figure 6. Strain amplitude dependence (a) and frequency dependence (b) of the shear dynamic moduli of alginate hydrogels and PNRAHCs.
between alginate hydrogels and PCL nano hydrogel composites higher shear loss modulus. It indicated that the PCL nano hydrogels and the nano fibers layers provided the alginate hydrogels with higher bearing capacity and better energy dissipation capacity. The PNRAHCs would be a good candidate to prepare scaffolds for fibrocartilage tissue engineering.

4. Conclusions

By combining alginate hydrogels with electrospinning PCL nanofibers, PCL nanofibers reinforced alginate hydrogel composites (PNRAHCs) were obtained. Coral-like and spider web-like microstructures were formed between alginate hydrogels and PCL nanofibers, and some PCL nanofibers formed bundles, which connected the hydrogels and the nanofibers layers. As a result, the PNRAHCs presented higher shear storage modulus and higher shear loss modulus. It indicated that the PCL nanofibers layers provided the alginate hydrogels with higher bearing capacity and better energy dissipation capacity. The PNRAHCs would be a good candidate to prepare scaffolds for fibrocartilage tissue engineering.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID iDs

Xufeng Dong https://orcid.org/0000-0001-5556-280X

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