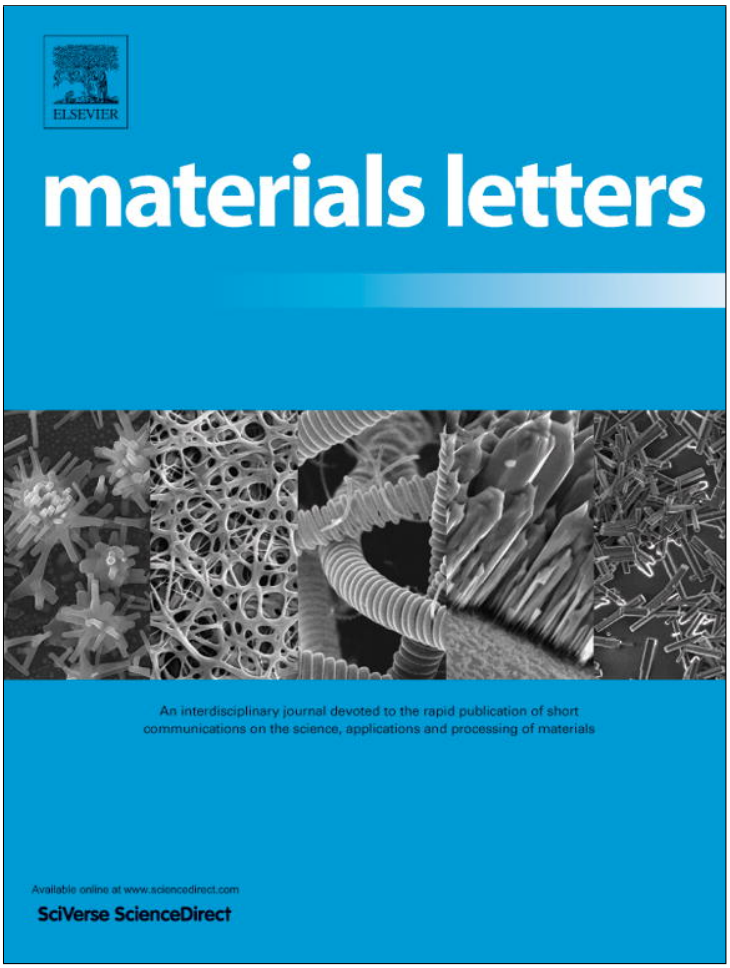

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Electrospun nanoyarn scaffold and its application in tissue engineering

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ABSTRACT

Silk fibroin (SF)/Poly(L-lactide-co-caprolactone) P(LLA-CL) nanoyarn scaffolds were prepared by dynamic liquid electrospinning. The scaffold morphology was observed by scanning electron microscopy (SEM) and mechanical properties of the scaffold were examined. L929 mouse fibroblasts were cultured on the nanoyarn scaffolds. Cell morphology, infiltration and proliferation on the scaffolds were investigated by SEM, hematoxylin-eosin (H&E) staining and methylthiazol tetrazolium (MTT) assay, respectively. The results indicated that cells showed an organized morphology along the nanoyarns and considerable infiltration into the nanoyarn scaffolds. It was also observed that the nanoyarn scaffold significantly facilitated cell proliferation. Therefore, this work provides a promising approach to fabricate scaffolds for tissue engineering applications.

1. Introduction

Recent approaches using electrospun scaffolds for tissue repair demonstrate the superiority of electrospinning in the fabrication of engineered scaffolds for tissue engineering applications [1]. Electrospun scaffolds have been widely investigated as substitutes for bone [2], cartilage [3] and tendon [4] regeneration. However, some limitations appeared in the applications due to the layer-by-layer process of electrospinning [5]. One of the problems encountered in the use of electrospun scaffolds is the limited infiltration of cells [6]. This is attributed to the smaller pore size of electrospun scaffold compared with that of cell diameter.

Many efforts have been addressed to improve pore size of the electrospun scaffolds so as to facilitate cell infiltration into electrospun scaffolds. One of the efforts relates to the optimizing of scaffold structures. For example, selecting of special materials for the scaffold fabrication was described to be a desirable approach to improve cell infiltration [7]. In this approach, water-soluble poly(ethylene oxide) (PEO) polymer was served as sacrificial fibers and leached out from nanofiber scaffolds to improve pore size. Leong et al. [8] reported a cryogenic electrospinning technique which utilized ice crystals as porogens to create larger pores within nanofiber scaffolds. Bryan et al. [5] designed a novel collection system for electrospinning to prepare cotton-like, uncompressed scaffold, which supported significantly enhanced cell in-growth penetration. However, the improved cell infiltration attained by those approaches inevitably compromise scaffold integrity or mechanical properties.

This study aims to fabricate a novel nanoyarn scaffold and evaluate its applications in tissue engineering. Electrospun SF/P(LLA-CL) nanofibers were deposited in a water vortex and bonded into nanoyarns and then collected by a rotating mandrel. The nanoyarn made up by nanofibers might have improved surface properties and porous structure. The nanoyarn scaffolds, composed of aligned nanoyarns, were seeded with mouse fibroblasts. The results showed that the nanoyarn scaffolds supported significantly accelerated proliferation, organized morphology and improved infiltration of cells compared with that of the random-oriented nanofibrous scaffolds.

2. Experimental

Materials: P(LLA-CL) (Mw=300 kDa; LA:CL=75:25) was obtained from Nara Medical University, Japan. Bombyx mori silkworm cocoons were kindly given by Jiaxing Silk Co. Ltd. (Jiaxing, China). L929 cells were obtained from institute of biochemistry and cell biology (Chinese Academy of Sciences, China). All reagents and medium for cell culture and seeding were purchased from Invitrogen.

Scaffold fabrication: Regenerative silk fibroin was prepared as our previously used method [9]. SF and P(LLA-CL) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol to obtain blended solution at the concentration of 8%(wt/v) with the SF/ P(LLA-CL) weight ratio of 10:90. A voltage of 15 kV was applied to a spinneret with a distance of 15 cm to the collecting target. The solution was fed at consistent rate of 1.0 mL/h. Electrospun nanofibers were

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deposited in the water vortex (Fig. 1) to prepare nanoyarns which were collected by the rotating mandrel to obtain the nanoyarn scaffold. For the fabrication of nanofiber scaffold, electrospun nanofibers were collected on a solid plate under the above mentioned electrospinning parameters.

Characterization: Scaffolds were characterized in terms of morphology and mechanical properties. Dry samples were sputter-coated with gold, and then observed by a scanning electron microscopy (SEM, Hitachi TM-100, Japan) at an accelerating voltage of 10 kV. Prior to the stress–strain test, the samples were maintained at an incubator with humility of 65% and temperature of 37 °C overnight. The test was performed using a universal materials tester (H5K-S, Hounsfield, UK) with a load cell of 50 N and a consistent crosshead speed of 10 mm/min. The ultimate tensile stress was calculated as the recorded force divided by the cross section of the scaffold and the strain was obtained as the ratio of elongation to the gauge length. For the nanoyarn scaffold, test was conducted in the directions parallel and perpendicular to the nanoyarns, as it was reported that the fiber alignment has a profound effect on scaffold mechanical properties [10].

Cell morphology and infiltration: L929 cells were maintained and expanded in high glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 units/mL streptomycin and incubated at humidified atmosphere with 5% CO_2 at 37 °C. A total number of 1.0×10^4 cells were seeded on the electrospun nanoyarn and nanofiber scaffolds in 24-well cell culture plates. The media was refreshed every other day. Cell morphology on the scaffolds was examined 4 days post-seeding. Briefly, cells cultured on the scaffolds were fixed by 4% paraformaldehyde for 30 min at 4 °C, dehydrated with gradient ethanol and followed by drying at room temperature overnight. And then the samples were sputter-coated with gold and observed by a SEM. Cell infiltration on the scaffolds was determined by paraffin-embedding H&E staining. After fixing by 4% paraformaldehyde, samples were treated by dehydration, clarification, infiltration and paraffin embedding. Cross-section samples (10 μm) were obtained and stained with hematoxylin and eosin and observed by an optical microscope (H600L, Nikon, Japan).

Cell proliferation: Cell proliferation was conducted using MTT assay at the time points of 1, 4 and 7 days post-seeding. Specifically, at each time point, cell culture medium was replaced by MTT solution in serum-free DMEM and maintained for 4 h at 37 °C to allow formazan formation. After the incubation, the accumulated
formazan was dissolved in dimethylsulfoxide (DMSO) and measured using a Multiskan Mirco-plate reader (MK3, Thermo, USA) at 492 nm ($n=5$).

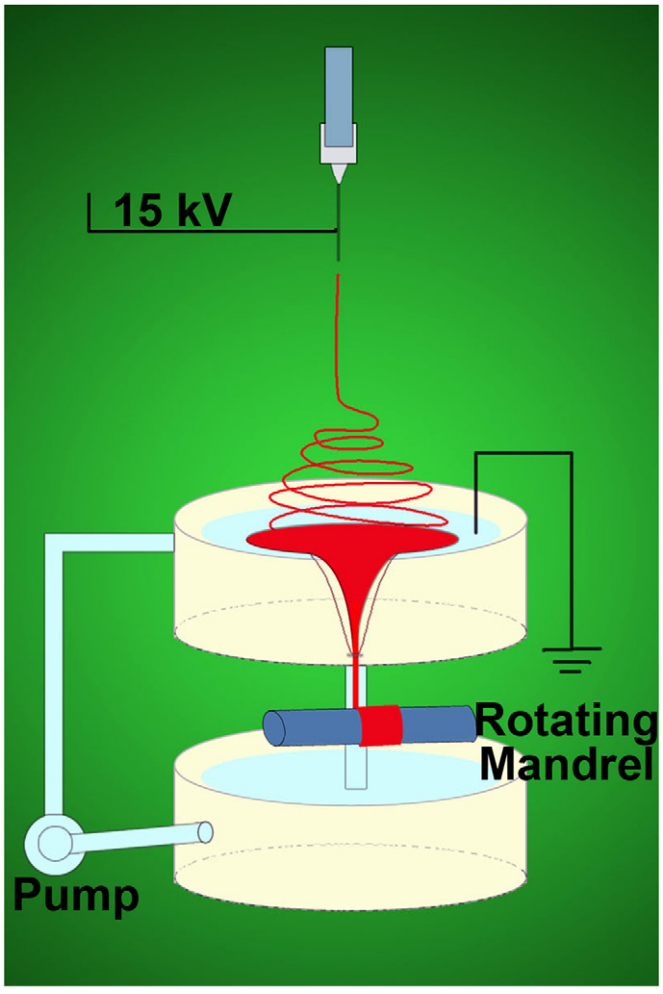

Fig. 1. Schematic of the dynamic liquid electrospinning system fabricates nanoyarn scaffold.

3. Results and discussion

The nanoyarn scaffold was prepared using a dynamic liquid electrospinning (Fig. 1). It is the vortex in water that twists electrospun nanofibers into nanoyarns. When the nanofibers arrive at the water surface, they flow with the whirly water in the vortex and form nanoyarns which are collected by the rotating mandrel. The obtained scaffold (Fig. 2A), made up of aligned nanoyarns (~20 μm), shows a porous structure with large pores (20–50 μm in diameter) and grooves on the surface. However, the nanofiber scaffold (Fig. 2B) has a smooth surface with pores (~5 μm) which might be smaller than cell diameter as reported by Blakeney et al. [5]. The nanoyarn scaffold shows similar mechanical properties with that of electrospun aligned nanofiber scaffolds in previous reports [10]. Typical stress–strain curve of the nanoyarn scaffold (Fig. 2C) illustrates significant differences of tensile strengths between the directions parallel (3.55 ± 0.21 MPa) and perpendicular (0.23 ± 0.04) to the nanoyarns ($p < 0.05$). However, the elongation of the nanoyarn scaffold in the perpendicular ($82.0 \pm 5.2\%$) direction is about two-fold greater than that in parallel ($43.5 \pm 3.36\%$) direction. Compared with the nanofiber scaffold (Fig. 2D), the nanoyarn scaffold shows a little weaker mechanical properties. This might be due to the highly porous structure of nanoyarn scaffold, as it was reported that a higher porosity compromises mechanical properties [11].

There is increasing reports about scaffold surface affects cell growth pattern in terms of adhesion, migration and differentiation [12]. Scaffold surface properties of alignment, geometric features [13] and roughness, for example, were demonstrated to regulation cell proliferation and differentiation.

In order to investigate scaffold surface effects on cell growth, morphology of the cells was observed by SEM. As shown in Fig. 3A, cells attached and grew well on the nanoyarn scaffold. It can be found that most of the cells exhibited an elongated morphology and show an organized growth pattern along the nanoyarns. In addition, migrating cells were observed to adhere tightly to the nanofibers between adjacent nanoyarns. This result indicates that the nanofibers between nanoyarns help cells bridging up, which might be contributed to functional tissue formation. In contrast, cells show random-oriented growth pattern on the nanofiber scaffold.

Image of H&E staining (Fig. 3C) reveals elongated nucleus phenotype and intensive cell infiltration throughout the nanoyarn scaffold. However, cells seem to be restricted to grow on the

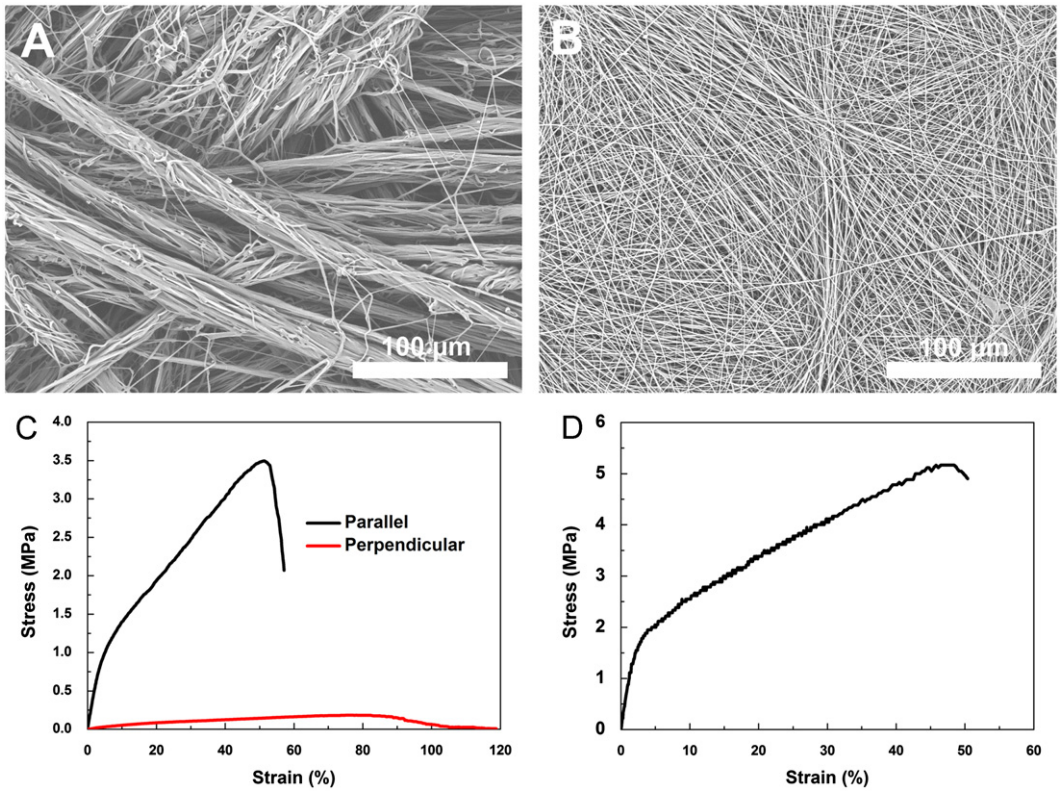

Fig. 2. Scaffold morphology of the nanoyarn (A) and nanofiber scaffold (B) observed by SEM. Typical stress–strain curves show that the nanoyarn scaffold (C) is a little weaker than the nanofiber scaffold (D) in mechanical properties.

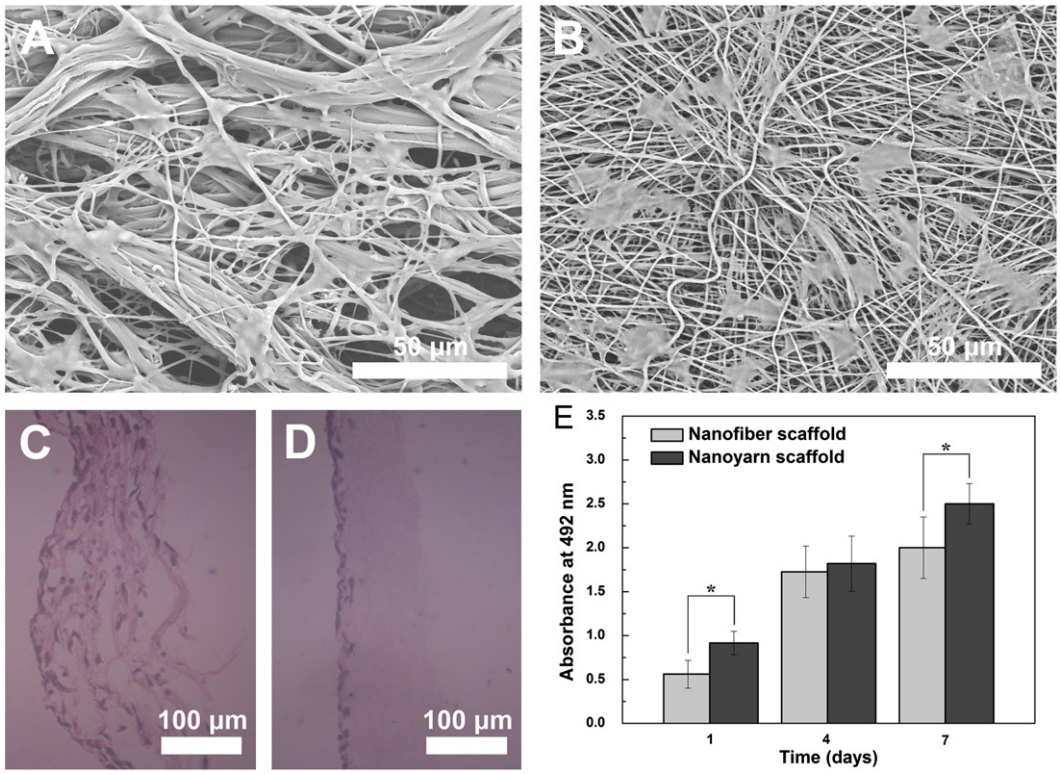

Fig. 3. SEM images of L929 cells on the nanoyarn scaffold (A) and nanofiber scaffold (B). H&E staining images reveal cell infiltration in nanoyarn (C) scaffold and nanofiber scaffold (D). Cell proliferation on the scaffolds was determined by MTT assay (E).

surface zone of the nanofiber scaffold (Fig. 3D). Improved cell infiltration indicates that the nanoyarn scaffold with larger pores significantly facilitates cell ingrowth compare with the nanofiber scaffold. In agreement with the H&E staining results, cells cultured on the nanoyarn scaffolds have a significantly accelerated proliferation rate than that on the nanofiber scaffold (Fig. 3E).

All the results imply that the nanoyarn scaffold has a positive effect on cell growth.

4. Conclusion

We have developed a novel nanoyarn scaffold by dynamic liquid electrospinning. The scaffold has excellent surface properties and porous structures. L929 cells show an oriented growth pattern and enhanced cell infiltration on the nanoyarn scaffold. This scaffold is beneficial for cell growth and may be served as tendon or cartilage substitutes in tissue engineering applications. Further studies will be focused on its implantation into animal models for the investigation of biocompatibility in vivo.

Acknowledgments

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