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Lab Note

Gelatin/hyaluronic acid nanofibrous scaffolds: biomimetics of extracellular matrix

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In living systems, extracellular matrix (ECM) plays a pivotal role in controlling cell behavior [1]. One of the most significant objectives in tissue engineering is to design and obtain scaffolds with the ability of biomimicking natural ECM in chemical compositions, physical structure, and biological functions [2,3]. Natural ECM is composed of a cross-linked porous network of multifibril collagens with diameters ranging from 50 to 500 nm and embedded in glycosaminoglycans [4,5].

Electrospinning technique has been recognized as an efficient processing method for the preparation of ECM analog scaffolds composed of nanoscale fibers, which possess high surface area to volume ratio and high porosity and thus can promote cell adhesion, migration, and proliferation [3]. Since gelatin (GE) is a protein biopolymer derived from partial hydrolysis of native collagens and hyaluronic acid (HA) is a kind of polysaccharide in natural ECM, electrospun nanofibrous scaffolds of GE and HA complex could biomimic both the composition and the nanofibrous structure of natural ECM for tissue engineering. Here, we report the preparation and preliminary characterization of GE/HA blended nanofibrous scaffolds.

GE/HA nanofibrous scaffolds were prepared through an electrospinning method [6]. Pure GE (type A; Sigma-Aldrich, St Louis, USA) and HA (sodium salt, MW = 200,000; Zhejiang Dali Technology, Hangzhou, China) were blended with different weight ratios (GE/HA = 10 : 0, 9 : 1, 8 : 2, 7 : 3 and 6 : 4) and then dissolved in 2,2,2-trifluoroethanol (TFE)/water (1 : 1; *v*/*v*) solvents and stirred at room temperature for 6 h. GE/HA ratios below 5 : 5 were not tested, because no fibers were formed due to high viscosity and surface tension of the blends solution. The concentration of solutions was set at 10% (*w*/*v*), because a small quantity of nanofibers with bead-on-strings occurred when concentrations were set <8% (*w*/*v*) or >12% (*w*/*v*) (data not shown). The solutions were placed into a 2.5-ml plastic syringe with a blunt-ended needle with an inner diameter of 0.21 mm. The needle was located at a distance of 13–15 cm from the grounded collector. A syringe pump (789100C; Cole-Parmer, Vernon Hills, USA) was employed to feed solutions to the needle tip at a feed rate of 0.8 ml/h. A high electrospinning voltage (20 kV) was applied between the needle and the ground collector using a high voltage power supply (BGG6-358; BMEI, Beijing, China).

The morphology of the electrospun fibers was observed with a scanning electronic microscope (SEM) (JSM-5600; JEOL, Tokyo, Japan). The diameter range of the fabricated ultrafine fibers was measured based on the SEM images using an image visualization software Image J 1.34s (National Institutes of Health, Bethesda, USA) and calculated by selecting 100 fibers randomly observed on the SEM images. Bead-on-strings occurs when pure GE is used. The nanofibers became uniform without bead-on-strings and the average diameters of nanofibers gradually increased with increasing HA content in the blends. The unusually high molecular weight of HA contributed to the distinctly increased viscosity of the mixed solution [7]. The SEM micrographs of GE/HA nanofibers with different weight ratios are shown in **Fig. 1**. The most appropriate GE/HA ratio was 7 : 3.

The GE/HA nanofibrous scaffolds were finally cross-linked in 1.5 M ethanol solutions of *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride at 4°C for different times, and then dried in a vacuum at room temperature for 3 days. The SEM micrographs of GE/HA nanofibrous scaffolds with different cross-linking time are shown in **Fig. 2**. The optimal cross-linking time was 30 min.

The surface wettability plays an important role in affecting cell attachment, proliferation, and migration [8]. To clarify surface wettability of electrospun GE/HA nanofibrous scaffolds, we measured the water contact angles of nanofibrous scaffolds before and after cross-linking (**Table 1**). Pure GE nanofibrous scaffolds were 88.3°. With the increase of HA content, water contact angle on nanofibrous scaffolds

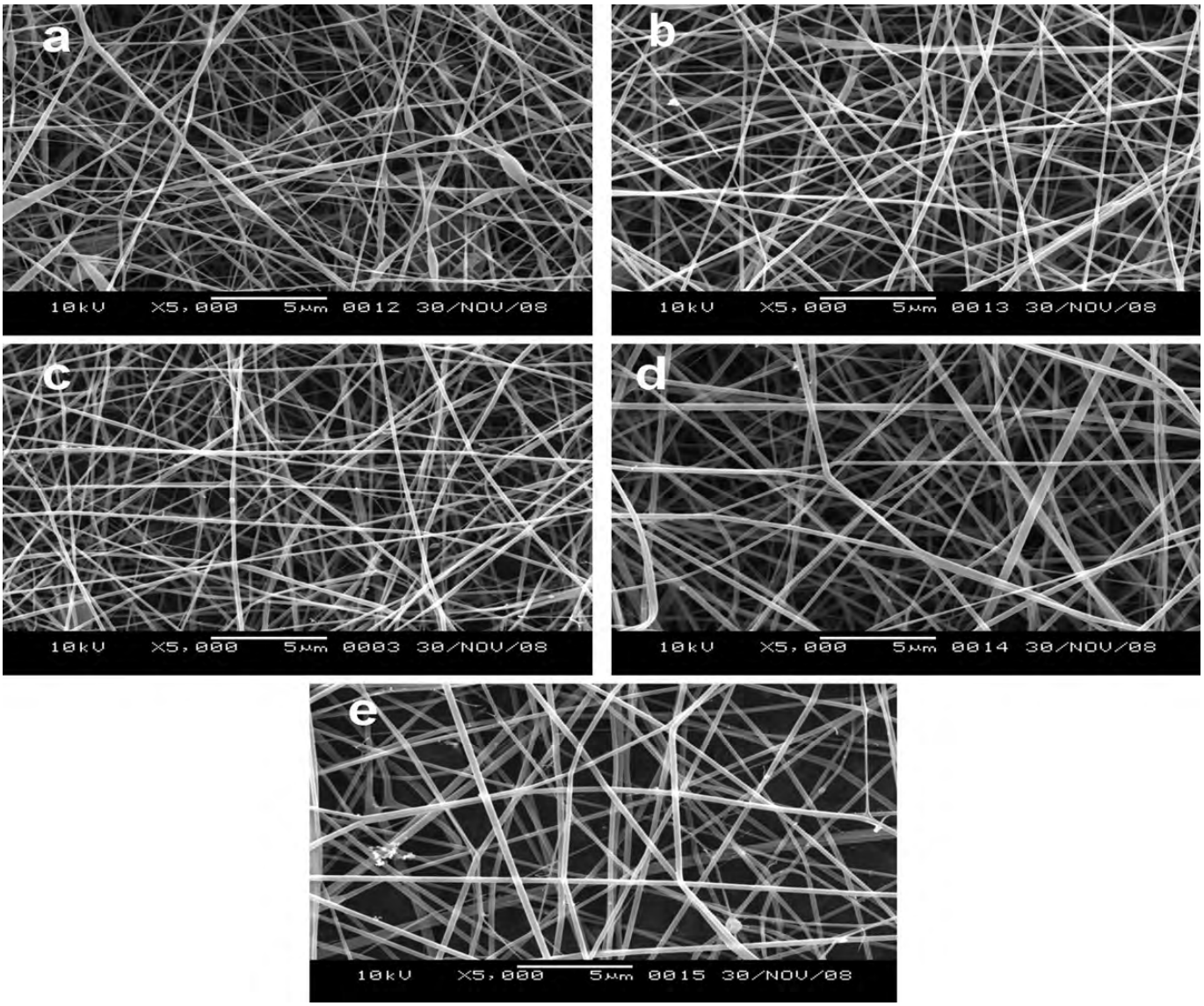

Figure 1 SEM images of GE/HA nanofibers with different weight ratios (a) 10 : 0, (b) 9 : 1, (c) 8 : 2, (d) 7 : 3, (e) 6 : 4.

obviously decreased. The results showed that the hydrophilicity increased and became ultra-hydrophilic along with the increase of HA content. Compared with non-cross-linked nanofibrous scaffolds, all the water contact angles increased to some extent after the nanofibrous scaffolds were cross-linked. But they were of good hydrophility.

To test the cell compatibilities of the nanofibrous scaffolds, fibroblast cells (L929s; Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in Dulbecco's modified Eagle medium (Gibco, Grand Island, USA) with 10% fetal bovine serum and 1% antibiotic–antimycotic in an atmosphere of 5% CO_2 and 37°C, and the medium was replenished every 3 days. The cross-linked nanofibrous scaffolds were prepared on circular glass coverslips (14 mm in diameter) and fixed the coverslips into 24-well plates with stainless steel ring. Before seeding cells, scaffolds were sterilized by immersion in 75% ethanol for 2 h, washed 3 times with phosphate-buffered saline (PBS) solution, and then washed once with the culture medium. For the proliferation study, fibroblast cells were seeded onto nanofibrous scaffolds and glass coverslips (control) ($n = 3$) at a density of 2.0×10^4 cells/ml for 1, 3, 5, and 7 days. Unattached cells were washed away with PBS solution and attached cells were quantified by MTT method. Statistical differences were determined by one-way analysis of variance and the differences were considered to be statistically significant at $P < 0.05$.

The L929s had good proliferation on the nanofibrous scaffolds (**Fig. 3**). Cell viability had no significant difference among nanofibrous scaffolds in comparison with coverslips (control) at Day 1. From Days 3 to 7, cell proliferation on pure GE and GE/HA nanofibrous scaffolds

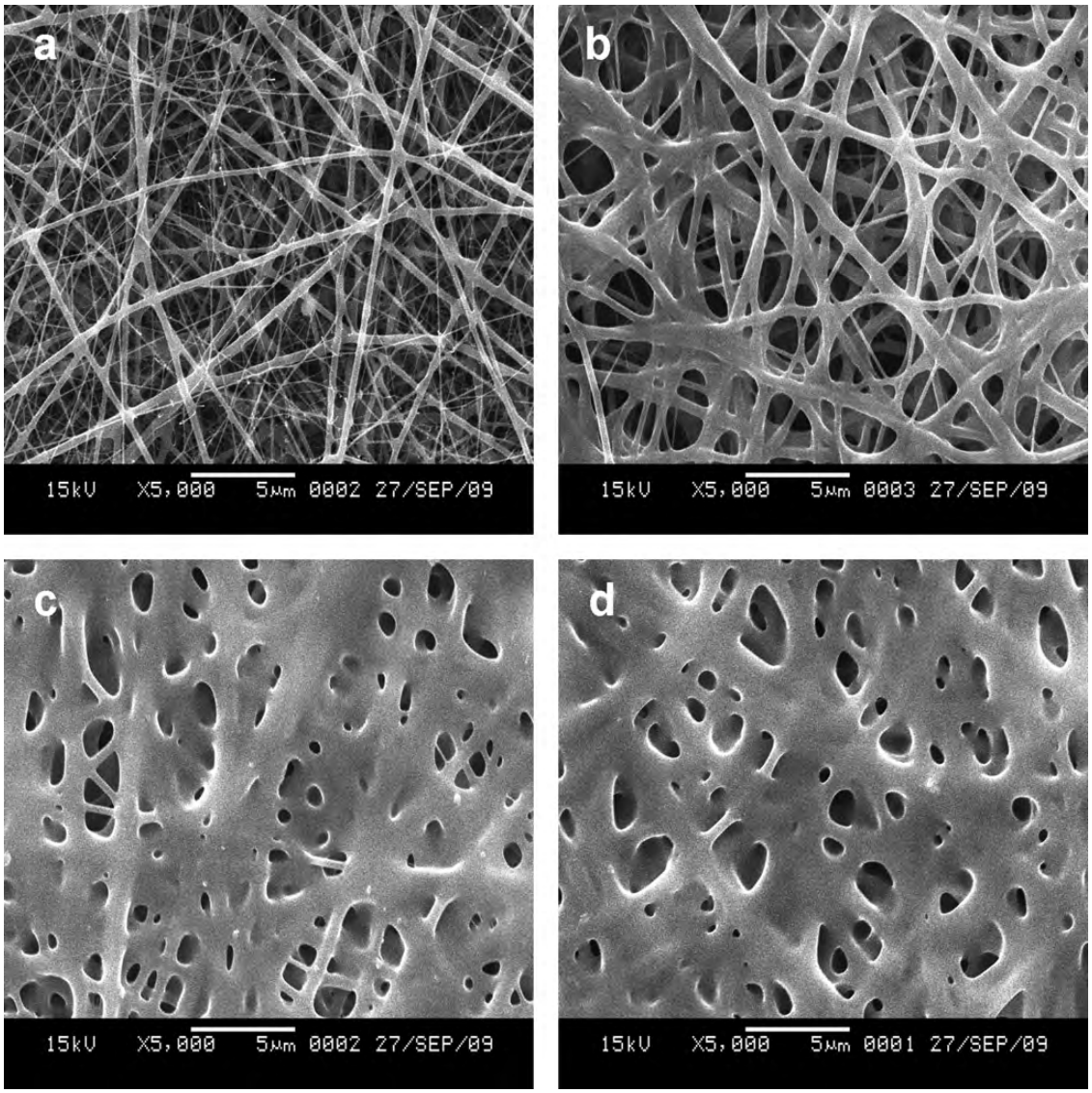

Figure 2 SEM images of GE/HA (6:4) nanofibrous scaffolds after cross-linking at different time points (a) 0 min, (b) 30 min, (c) 45 min, (d) 60 min.

Table 1 The water contact angle of electrospun GE/HA scaffolds before and after cross-linking

Weight ratios	Contact angles (°)	
	Non-cross-linked	Cross-linked
10 : 0	88.3 ± 1.8	99.9 ± 2.8
9 : 1	86.2 ± 1.5	90.3 ± 2.1
8 : 2	84.5 ± 1.7	87.4 ± 1.8
7 : 3	43.2 ± 1.4	83.3 ± 2.4
6 : 4	22.3 ± 1.3	72.5 ± 1.6

showed significant increase ($P < 0.01$) when compared with that on the coverslips, and the GE/HA (6 : 4) nanofibrous scaffolds showed the most significant increase.

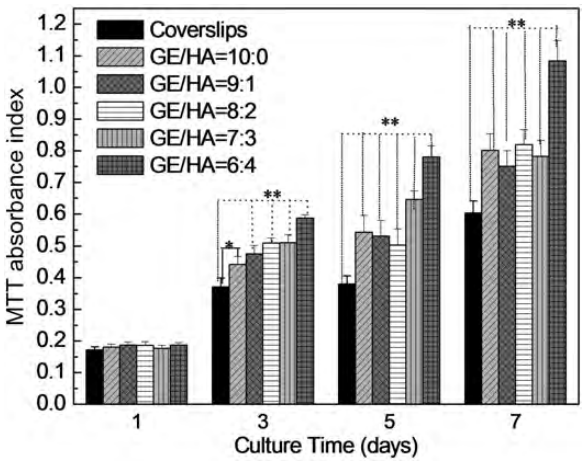

Figure 3 Proliferation of L929s cultured on GE/HA nanofibrous scaffolds, coverslips for 1, 3, 5, and 7 days $*P < 0.05$; $**P < 0.01$.

In summary, we have successfully prepared GE/HA nanofibrous scaffolds by an electrospinning method. The prepared GE/HA nanofibrous scaffolds possessed good hydrophility and cell compatibility *in vitro*. Further work will focus on evaluating their biocompatibility and biodegradability *in vivo*. Furthermore, the cross-linked GE/HA nanofibrous scaffolds will develop tissue engineering scaffolds for skin tissue regeneration or wound dressing.

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