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Hydrodynamically Stable Adhesion of Endothelial Cells on Gelatin Electrospun Nanofibrous Scaffolds

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Abstract

In the current research, electrospun gelatine fibers for vascular tissue engineering was successfully fabricated with the aim of increasing the adhesion and stability of cells under shear stress. The gelatin solutions were prepared with different amount of gelatin (10, 15, 20 and 25% wt) which dissolved in different ratios of co-solvent acetic acid / water (1:9, 1:3 and 1:1). The experiments showed the best conditions for electrospinning of the gelatin solution in where the concentration was 25% wt gelatin while it was dissolved in the ratio of 1:9 (v/v) water/ acetic acid and the applied voltage was 23 or 25 kV. The SEM images revealed the average size of diameter were about 190 and 210 nm, respectively. For studying the cell adhesion properties of fabricated scaffolds under shear stress, a parallel plate flow chamber with different flow rates was used. Here, Human Umbilical Vein Endothelial Cells (HUVECs) were either maintained in static condition or exposed to laminar shear stress (48 ml/min) for 12 h. This study shows that the constructed gelatin scaffold is remarkably suited for stable adhesion of cells under shear stress.

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Keywords: Gelatin, Electrospinning, Fibrous scaffold, Shear stress, Tissue engineering

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1. Introduction

During the last decade, the development of nanotechnology was led to evolution in many applied scientific fields especially in tissue engineering. The main consideration of researches in this area is fabrication of suitable scaffold that is able to mimic the natural structure, extra cellular matrix (ECM). Ideally a scaffold should possess the following characteristics to bring about the desired biological response: (1) interconnecting pores of appropriate scale to transfer nutrient and waste materials, (2) biodegradable and biocompatible, (3) appropriate surface chemistry to favor cellular attachment, differentiation and proliferation, (4) adequate mechanical properties to match the intended site of implantation and handling, (5) not induce any adverse response and, (6) be easily fabricated into a variety of shapes and sizes.

Among different methods of scaffold fabrication, it seems that electrospinning is more acceptable for scaffold production. Unique characteristics of electrospun fibers that consist of high surface area to volume and high density of pores have resulted in using them as scaffolds for cell/tissue culture [1, 2]. In order to use polymeric scaffold in tissue engineering, the most important characteristics that should be targeted are biocompatibility and mechanical performance. In comparison with synthetic polymers, natural biopolymers generally have better biocompatibility and therefore are more suitable for human body. However, electrospinning of natural biopolymer is usually more difficult than a synthetic polymer. Among the natural polymers, gelatin is a very good one for cells adhesion and proliferation but due to forming a colloidal sol around or above 37°C for aqueas gelatin solution, the process of electrospinning is not easily possible [3-5].

In this paper, co-solvent water/ acetic acid was prepared and the effect of water and acid ratio on electrospinning of gelaion solution was investigated to produce suitable scaffold as a vascular graft. It’s worth to mention that blood vessels are constantly exposed to hemodynamic forces in the form of cyclic stretch and shear stress due to the pulsatile nature of blood pressure and flow [6, 7]. Therefore the scaffold used for this application should possess very good hydrodynamically stable cell adhesion properties. For monitoring of this characteristic, HUVECs seeded on electrospun gelatin scaffold, exposed to fluid flow shear stress in a flow chamber bioreactor.

2. Materials and Methods

2.1. Electrospinning of Gelatin Solution

In order to prepare solution for electrospinning gelatin fibers, gelatin (from porcine skin) and acetic acid were purchased from Fluka and Merck respectively. The distilled water produced by Fatee Electronic 6004. Different amounts of gelatin (10, 15, 20 and 25 gr) dissolved in 100 cc solvent which is consisted of acetic acid and distilled water with the ratio of (9:1, 3:1 and 1:1 v/v%) at 37°C at a vigorous stirrer for 3 h. Various prepared solutions are mentioned in the table 1.

The experimental setup was made up of a syringe pump (SP-500, JMS, Japan), a power supply, and a piece of flat aluminum foil as a collector which was placed in front of the syringe pump. A 20 ml plastic syringe with a needle size of 0.711 mm in diameter was filled with the solution. The tip of the needle was connected to the anode of high-voltage supply, and the cathode was clamped to the collector. The distance between the tip of the needle and collector, and the solution feeding rate were constant 15 cm and 0.5 ml/h respectively in all experiments, while the voltage was changed between 21, 23, 25 and 27 kV. Scanning electron microscopy (SEM) (MV2300) at an accelerating voltage of 15 kV was employed to study the morphology of produced fibers.
Table 1. The conditions of prepared solutions and electrospinning parameters

<table>
<thead>
<tr>
<th>number</th>
<th>Gelatin (10% wt)</th>
<th>Water/acid acetic (%v/v)</th>
<th>Voltage (kV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>(1:9)</td>
<td>21</td>
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<tr>
<td>2</td>
<td>15</td>
<td>(1:9)</td>
<td>21</td>
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<td>3-1</td>
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<td>20</td>
<td>(1:9)</td>
<td>25</td>
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<td>3-7</td>
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<td>4-5</td>
<td>25</td>
<td>(1:3)</td>
<td>27</td>
</tr>
</tbody>
</table>

2.2. Applying Shear Stress on Endothelial Cells

HUVECs (C554-Pasteur Institute of Iran) were seeded and cultured in static media (DMEM supplemented with 10% fetal bovine serum). Cells up to the third passage were used for all experiments. Electrospun samples were first cleaned three times with deionized water followed by isopropanol rinsing, then stored under vacuum overnight. Samples were then rinsed three times with PBS. Specimens measuring 2 cm×2 cm were placed on a circular scaffold holder in each well of 12-well plate and were soaked in culture medium overnight in order to facilitate protein adsorption and cell attachment. Monolayers were prepared by seeding HUVECs on prewetted scaffolds. After a seeding time of 1–2 h, each culture well was topped up with enough culture medium and incubated for 24–48 h until confluent. One of the seeded samples placed in a flow chamber and exposed to 48 ml/min fluid flow using a peristaltic pump (Dynamax Model RP-1 Peristaltic Pump).

Cell viability on the scaffolds was assessed by using acridine orange-propidium iodide (AO/PI) staining. Briefly, the stock solution (AO: 670 mmol/L, PI: 750 mmol/L) was prepared with PBS solution and kept in the dark at 4°C. Just before use, 0.01 ml AO and 1.0 ml PI were mixed and diluted by 10 times with PBS solution. The scaffolds cultured with endothelial cells were incubated with the AO/PI mixture for 10 min and observed under a fluorescence microscope. Live cells were stained in green (AO) whereas dead cells were colored red (PI).

3. Results and Discussion

3.1. Optimum condition for fabricating suitable gelatin fibers

As shown in figure 1, in 10% and 15% wt concentration of gelatin, no fibers created due to low concentration of solutions. When the jet leaves the needle tip during electrosprinning, the polymer solution is stretched during traveling towards the collection plate, the involved molecule chains prevent the driven jet to be broken up and maintain a continuous solution jet. In contrast, in low concentration or low viscosity of polymer the molecule chains can’t form the stable jet and as-spun fibers.
The gelatin solution concentration increased to 20% wt while prepared by three different ratios of solvents. The fibers weren’t uniform in the tests number 3-1 to 3-6. Also the stable jet didn’t form in the tests number 3-7 to 3-9; therefore the gelatin fibers weren’t fabricated.

As shown in the figure 2, there are a lot of beads in the test 3-1. It may be because of surface tension which has an effect on decreasing the surface area per unit mass of a fluid. In a high concentration of free solvent molecules, there is a greater tendency for the solvent molecules to congregate and adopt a spherical shape due to surface tension. Sometimes in electrospinning process the higher voltages can overcome surface tension but
increasing the voltage wasn’t effective on the test 3-1 to 3-3. While in the tests 3-4 to 3-6 the formation of beads decreased by increasing voltage which is shown in the figure 3-1 and 3-5.

Reasons why the stable jet was not formed during tests 3-7 and 3-8 were higher surface tension which was due to increasing the amount of water and high electrostatic force couldn’t affect the surface tension forces.

Figure 3 related to the tests 4 revealed that the as-spun fibers with higher concentrations of gelatin formed more uniform and smooth fibers than the later tests. In the tests 4-2 and 4-3 a very stable jet was formed and the results of SEM images were favorable. In the test 4-4 and 4-5 a few beads were noticeable in the SEM images.

The main reason for two latter ones was the increasing of solvent surface tension due to water increasing and its effect on the fibers morphology as mentioned above. But by increasing the applied voltage in each series of tests, the average diameter decreased as the average diameter for the test 4-2 and 4-3 was about 210 and 190 nm and for the tests 4-4 and 4-5 was 220 and 210 nm, respectively. The higher voltage has been led to more stretching and acceleration of the jet solution which left the tip of the needle as a result of greater electrostatic forces which is exerted the jet. This can influence on reducing the diameter of the fibers and also encourage faster solvent evaporation to yield drier fibers.

3.2. Cell Experiment Analysis

By the help of computational fluid dynamic it is understood that fluid flow of 48 ml/min will result in better culture and growth of ECs. By running the system cells in comparison with the control system are firmly attached and after 12 hr fluid flow they are not only detached but also started to elongate which is a great response to the flow passing over them.

The AO/PI staining showed that endothelial cells cultured onto gelatin electrospun scaffold, attached, survived and proliferated very well, confirming the advantages offered by a composite natural matrix in providing appropriate biocompatibility. In addition, Fig. 4 depicts that flow rate of 48 ml/min results in not only detrimental effects on efficacy of attachment on the scaffold but it also helps cells be mechanically stimulated for further mechanobiological studies.

![Fig. 4](#)

Fig. 4. The AO/PI staining showed that the HUVECs remained (a) >75% viable on the gelatin scaffold after cultured 1 day statically in vitro and (b) >90% viable on the gelatin scaffold after cultured 12 hours dynamically in vitro (AO: green; PI: red).

4. Conclusions

The purpose of this paper was fabrication of electrospun gelatin fibers. As gelatin is one of the important natural polymer that has similar characteristic to collagen, many researchers tried to obtain the best condition for electrospinning of gelatin solution. Gelatin is soluble in some toxic solvents; therefore preparation of
gelatin solution with water-based solvents have been drawn attentions and this paper has been attempted to explore the effect of water/ acetic acid as a co-solvent. The gelatin solutions were prepared with different amount of gelatin (10, 15, 20 and 25% wt) which dissolved in different ratios of co-solvent water/acetic acid (1:9, 1:3 and 1:1). Also various voltages applied for desired solutions to obtain the optimum voltage. The experiments revealed the best conditions for electrospinning of gelatin solution in where the concentration was 25% wt gelatin while it was dissolved in the ratio of 1:9 (v/v) water/ acid acetic and the applied voltage was 23 or 25 kV and the average diameter was about 210 and 190 nm, respectively. In addition, cell experiments with applying shear stress in a flow chamber bioreactor proved that, the prepared fibrous gelatin scaffold using this method can be utilized in vascular tissue engineering applications and is suitable for cell adhesion and cell culture under hydrodynamic forces.

References