Biomimetic Nanofiber by Electrospinning of Snail Mucus

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**Abstract:** Background: Snail Mucus (SM) is secreted by the pedal gland of snails, and has a fibrous structure when it crawls upside down on the plane. It contains biologically active compounds that perform medical functions, such as glycol acid, natural antibiotics, and glycoprotein.

**Methods:** For this paper, we prepared fibers using electrospinning to simulate this natural fiber for the first time, and we can produce the nanofiber with mucus from a snail. The effects of dissolution time and the spun solution were also investigated.

**Results:** The results show that biomimetic nanofibers with different diameters can be obtained using electrospinning. When the concentration of the spun liquid was increased from 6 wt% to 8 wt%, a fiber with about a 200 nm diameter can be obtained. The adjustment of the concentration plays a crucial role in electrospinning.

**Conclusion:** The investigation and utilization of biomimetic nanomaterials can promote the development of tissue engineering effectively.

**Keywords:** Snail mucus, biomimetic, biomaterial, electrospinning, nanofiber, glycol.

1. INTRODUCTION

Nanotechnology is an evolving technology that has been identified as invaluable. Electrostatic spinning is one of the most popular and effective methods for preparing nanofibers. Electrostatic spinning, in the high-pressure electrostatic solution with polymer solution spinning process, can be prepared to a diameter of tens to hundreds of nanometers of fibers. The product has a high porosity and a large surface area, even lying in diameter. In the biomedical [1-4], environmental engineering [5, 6] and textile fields, it has a high application value. For example, Yu [7] uses the entire snail body mixed with PVA to prepare the spun solution for electrostatic spinning, and the obtained nanofibers have rough surfaces. Tian et al. [8] extracted protein from muskels and mixed it with PVA to create a uniform sea-silk solution. The results showed that the concentration of the solution was closely related to the fiber diameter. Zhou et al. [9] placed the silkworms in alcohol to make a homogenous solution and mixed them with PVA. The effects of the spinning process on the fiber diameter and fiber surface were studied. However, one cannot ignore the fact that the preparation of the solution needs PVA. This kind of water-soluble polymer material improves the spinnability of the solution, that is to say, a single protein solution cannot be spun. This experiment uses the natural mucus secreted by snails to explore snail mucus spinnability.

Snail mucus is derived from the snail's abdominal foot gland secretion. It consists of many substances, such as glycoprotein, natural allantoin, collagen, natural antibiotics, glycol acid, vitamins, calcium salts, etc. [10-13]. In ancient Greece, Hippocrates used snail mucus mixed with milk for the medical treatment of skin scars. The unique advantages of the pedal mucus have been proved, showing that it can promote the treatment of injured tissues and wounds [14-18], and this has gained the attention of many researchers [19]. In recent years, extracted snail mucus has also been used in the cosmetics industry. We found that snails crawl on the 180° inverted plane in the secretion of fibrous sticky stria [20], indicating that snail mucus has a certain degree of spinning. Directly obtained or fresh snail mucus cannot be used after one week. Freeze-dried snail mucus can retain its bioactivity for long shelf life characteristics, but it does not maintain a stable structure, and cannot, therefore, be applied directly. To solve this problem, we used a method of electrostatic spinning to biomimic the natural fiber of snail mucus.

Using the biomimetic of snail mucus, the snail mucus itself can promote cell repair and growth, and the nanofiber membrane has the structure and biological function of a bionic extracellular matrix (ECM) to a certain extent. This provides an ideal microenvironment for cell adhesion, proliferation and differentiation. It provides a potential candidate for medical applications based on natural materials and biomaterials. We also explored the effects of the spun solution, concentrations and dissolution times on electrospinning.
2. EXPERIMENTAL SECTION: MATERIALS AND METHODS

The white jade snail (*Achatina fulica*) was purchased from the Shanghai Xingnong Ecological Park for use in the present study. The snails were first cultured in the laboratory at 25°C, 65% RH for about 24 h. After they were stabilized, the bodies were washed with deionized water to remove impurities. Then they were left to crawl on the inverted plane so that the mucus could be collected for sample A. The hydrated mucus samples were gathered by scraping the deposited mucus from the pedal gland. The freeze-dried powder was then stored at -20°C after centrifugation and freeze-drying. The snail lyophilized powder was dissolved in formic acid (Shanghai Lingfeng Chemical Reagent Co., Ltd.), and the spun solution was stirred at room temperature for 4 h. The dissolution time and spun solution concentration of spun solution are summarized in Table 1.

Table 1. List of dissolution time and concentration of spun solution.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Dissolution Time/h</th>
<th>Spun Liquid Concentration/wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM(B)</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>SM(C)</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>SM(D)</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

The different spun solutions obtained were transferred to syringes with a capacity of 10ml, respectively for electrospinning. A stainless steel Taylor cone was used, with an inner diameter of 0.8G. The distance between the needle and the receiving device was 15-18cm. The spinning voltage was gradually increased to 25kv at 20°C, 35%-45% RH. The morphology of the spinning results was observed using a field emission scanning electron microscope (SEM, Hitachi S-480). Prior to imaging, the samples were randomly selected and cut to 5 mm x 5 mm, attached to a sample stage, and then sputter-coated with gold.

3. RESULTS AND DISCUSSION

The fiber felt was composed of microfiber or nanofiber that had an excellent specific area that could be produced using electrospinning. Based on this technology, biomimetic nanofibers produced by electrospinning snail mucus can improve the effectiveness of wound dressings. Formic acid was used as a solvent to dissolve different concentrations of snail mucus for electrostatic spinning. Formic acid is a weak acid that is not completely ionized in water. Formic acid has the function of an ion-pairing agent. It increases the solubility of the protein and is more conducive to the preparation of the spun solution. During the spinning process, a nanofiber membrane free of formic acid can be obtained, as formic acid is extremely volatile. Thus, a variety of products can be provided for medical applications based on snail mucus.

Fig. (1) illustrates the morphological structure of the mucus sample obtained from a snail crawling on an inverted plane (Fig. 1A). Snail mucus can be provided with spinability. Fig. (1B) shows an SEM image of the nanofibers prepared from a spun solution with a concentration of 6wt%. It can be seen by using electrospinning, one can successfully bionically produce the fiber structure of snail mucus. The fibers were 700 nm in diameter, which was about ten times smaller than those observed in the snail crawling. It is shown in Fig. (2) that the peak positions of the sugar C-OH...
exhibit the stretching vibration absorption peak of OH in the range of 3570–3050 cm⁻¹ [21]. Amide I (1700–1600 cm⁻¹) and amide II (1600–1500 cm⁻¹) [21] are the characteristic peaks of the secondary structure of the protein. According to Fig. (2), all have characteristic peaks in the range of 1640–1660 cm⁻¹ and 1535–1550 cm⁻¹, and the protein secondary structure belongs to α-helix and random coil. As shown in Fig. (1C), with the prolongation of dissolution time, nanoparticles adhere to the surface of the fibers. The nanoparticle is known as calcium formate (CaFo) [22], it is the product of a chemical reaction between calcium carbonate [11, 12] in the snail mucus and the formic acid. It should be noted that the fiber diameter did not change significantly. When all other things were equal, increasing the dissolution time did not change the viscosity of the spun solution. If all other things including dissolution time, were equal, when the concentration of spun liquid was increased from 6wt% to 8wt%, a fiber of about 200 nm diameter could be obtained using electrostatic spinning (Fig. 1D). Although when the spun concentration and the solubility of snail mucus in formic acid were increased, the viscosity decreased, and was easily recognizable, so it was easy to obtain a fiber with a smaller diameter. By increasing concentrations, a solution that contained more ions of snail mucus had an increased conductivity effect. This could be the reason for the reduction in the fiber diameter.

Fig. (2). (A) FTIR image of a snail crawling on an inverted plane to secrete mucus. (B-D) FTIR images of SM nanofibers prepared using a spun solution with different concentrations and dissolution times. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

CONCLUSION

The investigation and utilization of biomimetic nanomaterials can promote the development of tissue engineering effectively. In this study, the fiber structure of snail mucus was made bionically for the first time. Biomimetic nanofibers created by electrospinning snail mucus can have different diameters by adjusting the concentration of the spun solution. These could provide a variety of products for medical applications based on snail mucus.

CURRENT & FUTURE DEVELOPMENTS

Snail mucus bionic nanofibers have made preliminary progress and have the potential for future biomedical applications.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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