Experimental Cell Research 338 (2015) 261–266

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

A novel bioactive membrane by cell electrospinning

Haiping Chen [a,b], Yuanyuan Liu [a,c,*], Qingxi Hu [a,c]

[a] Rapid Manufacturing Engineering Center, Mechatronic Engineering and Automation of Shanghai University, Shanghai 200444, PR China
[b] School of Mechanical and Electrical Engineering, Jinggangshan University, Ji'an 343009, China
[c] Shanghai Key Laboratory of Intelligent Manufacturing and Roboties, Shanghai 200444, China

ARTICLE INFO

Article history:
Received 30 June 2015
Accepted 17 August 2015
Available online 18 August 2015

Keywords:
Cell electrospinning
Bioactive membrane
Cell viability
Cell compatibility

ABSTRACT

Electrospinning permits fabrication of biodegradable matrices that can resemble the both scale and mechanical behavior of the native extracellular matrix. However, achieving high-cellular density and infiltration of cells within matrices with traditional technique remain challenging and time consuming. The cell electrospinning technique presented in this paper can mitigate the problems associated with these limitations. Cells encapsulated by the material in the cell electrospinning technique survived well and distributed homogenously within the nanofibrous membrane, and their vitality was improved to 133% after being cultured for 28 days. The electrospun nanofibrous membrane has a certain degradation property and favorable cell–membrane interaction that supports the active biocompatibility of the membrane. Its properties are helpful for supporting cell attachment and growth, maintaining phenotypic shape, and secreting an ample amount of extracellular matrix (ECM). This novel membrane may be a potential application within the field of tissue engineering. The ability of cell electrospinning to micro-integrate cells into a biodegradable fibrous matrix embodies a novel tissue engineering approach that could be applied to fabricate a high cell density elastic tissue mimetic.

1. Introduction

The cells of human's tissue are all embedded within 3-D structures, and they receive signals from surrounding areas. This environment can provide highly favorable condition for cells' growth, such as balanced nutrition and exchange of matter and energy, making cells constantly proliferating and secreting ECM.

In order to construct the same micro environment in vitro, combining cells, growth factors, and an ECM-like structure into one organic coalition to obtain some degree of biological function elicited by their interaction is a unique challenge to overcome. Nanofiber membranes can imitate the properties of cells' natural ECM, including high porosity and connectivity [1–2]. In addition, they possess some degree of similar mechanical properties, which favors the cell's adhesion, growth, migration and secretion of ECM [3]. However, the bore diameter of this membrane is too small and thus hinders the cells' ability to grow towards the inside of membrane, [4] cell migration, and cell proliferation [5].

Many scholars are studying how to inoculate cells uniformly into the inner porous areas of 3D scaffold structures. The current methods are the static cultivation by adding the cell suspension directly to the surface of the material [6–10], dynamic chamber seeding within complex bioreactors [11–14], and rotational vacuum seeding within complex bioreactors [15–16]. Though the methods for cell cultivation have had some progress, there are still some drawbacks: (1) The cells inoculated by static cultivation migrate and grow into the inner area from the scaffold surface, and the unidirectional migration and growth affect the interactions of the cell–cell and the cell–scaffold and the balanced development of the organization. (2) The cells introduced by dynamic cultivation are generally low-density, thus the growth of the new organization is slow. (3) Neither static cultivation nor dynamic cultivation can simulate the force of electromagnetic fields for some special organs (such as myocardial, nerves, etc.) in which the cells grow along a specific direction and obtain corresponding physiological functions [17–19].

The cell electrospinning technology [20], which incorporates living cells into the material with the well-established electrospinning technique, can achieve high density cells which are well-distributed throughout the nanofibrous membrane structure and scattered evenly in the membrane. This paper formed the nanofibrous membrane with cells by the cell electrospinning process, studied the effects of the electrospinning process on cell morphology, distribution in the membrane, vitality, and interaction with the membrane in vitro. The experimental results show that the cells achieve high activity, proliferate quickly, and grow well within the membrane. These results suggest that the development

* Corresponding author at: Rapid Manufacturing Engineering Center, Mechatronic Engineering and Automation of Shanghai University, Shanghai 200444, PR China. Fax: +86 021 66133296.
E-mail address: Yuanyuan_liu@shu.edu.cn (Y. Liu).

http://dx.doi.org/10.1016/j.yexcr.2015.08.007
0014-4827/

of a fibrous membrane by the cell electrospinning process may be useful for tissue engineering applications.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA, MW=2000, Aldrich) was dissolved in deionized water and heated with stirring to prepare the 8.8% PVA solution, which was then sterilized with by autoclave.

2.2. Isolation and culture of ASCs

ASCs were isolated from the adipose tissue. Adipose tissue was washed three times with phosphate-buffered saline (PBS, pH7.4) and treated with 0.075% type I collagenase (Washington Biochemical Corp., USA) at 37 °C for 30 min. Enzymatic activity was neutralized with Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY), containing 10% fetal bovine serum (FBS, HyClone, USA). The solution was then centrifuged at 1200 g for 10 min. The yielding cells were resuspended in the regular medium and plated at 4×10^4 cells/cm^2 in φ100 mm culture dishes (Falcon, USA). The dishes were then maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2 with the media changed twice a week. When having reached 70–80% confluence, cells were passaged and ASCs prior to passage 3 were used in the following study.

2.3. Preparation of bioactive membrane

To verify the validity of the cell electrospinning technique (experimental), cell electrospraying (control) was used for comparison.

The cell electrospinning parameters were as follows: the electrostatic field was 6.5 kV and the distance between the cathode and anode was 8 cm. Steady flow of the PVA solution mixed with 100 ul cell suspensions at a density of 5.0×10^7 cells/ml was achieved using a micro-pump, which was operated at a flow rate 160 ul/min. The fibers were collected on sterile plate (charged at 0 KV).

An apparatus, described in the document [5], was copied to produce the control membrane. 5×10^7 cells/ml of suspension was pushed into a sterile stainless steel needle tube (inside diameter 0.6 mm, charged at 20 KV) through sterile tubing with a micro-pump at a rate of 250 μl/min. At the same time, the PVA solution was forced at a rate of 160 μl/min into a capillary that charged at 6.5 KV. The distance between the cathode and anode was 8 cm, and the collecting plate at the cathode (charged at 0 KV) was moving on an x–y motion platform at the speed of 10 cm/s.

The experimental groups of membranes were placed in culture dishes with 8 ml of the growth medium, and the media was changed twice a week. The control groups of membranes were placed in culture dishes, incubated for 4 h to allow for cell adhesion to the membrane, and then 8 ml of the growth medium was added to each plate. The membranes were cultured in vitro in media changed twice a week.

2.4. Analysis of cell morphology and distribution

To visualize the morphology and distribution of ASCs, the two groups of membranes were submerged in fluorescent 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) dye (Molecular Probes) at 37 °C for 20 min following the manufacturer's protocol, and then they washed three times with PBS and visualized by fluorescence microscopy.

2.5. Cell–matrix interaction

After being incubated for 7 and 28 days, the two groups of membranes were fixed with 4% paraformaldehyde and then dehydrated. The samples were sputter-coated with gold and observed by scanning electron microscopy (SEM SU1510, Analysis and Testing Center, Shanghai University).

2.6. Superoxide dismutase (SOD) assay

After the two groups of membranes had been cultured for 28 days, the solution in the dishes were collected and centrifuged at 1000 g for 5 min. The supernatant was then retracted to assay SOD that had been secreted by cells using the kit following the manufacturer's protocol (n=3 per period).

2.7. Cell proliferation assay

The number of cells on the membrane was detected by DNA assays at 1, 7, 14 and 21 days. The membranes collected at different time points were crushed and then frozen and thawed repeatedly to release DNA. DNA quantification was performed (n=9/group/time point) using Hoechst 33258 dye (Sigma-Aldrich) following the manufacturer's protocol.

2.8. Degradation property

To explore the degradation property of the membrane, the membrane without cells was weighed after vacuum freeze-drying. The experiment group membrane and the control group membrane were placed in culture medium and then incubated in 37 °C carbon incubator for various periods of time. The two groups of membranes were taken out at the end of each of the degradation periods and washed thoroughly with distilled water and then dried. The membranes were weighed after vacuum freeze-drying (n=3 per period). The degradation rate of each membrane was calculated according to the following equation:

$$\text{Quality degradation}(\%) = \frac{W_2 - W_1}{W_1} \times 100\% \quad (1)$$

where W_2 and W_1 represent the weight of the membrane after and before degradation, respectively.

2.9. Statistical analysis

Statistical analysis was performed with GraphPad Prism software. A two-way ANOVA of data was done to compare degradation rate of membranes (n=3). T-tests of data were done to compare OD values at different time points (n=9). A two-way ANOVA of data was done to compare SOD content (n=3). P-values of less than 0.05 were considered statistically significant. All values are reported as means ± standard deviation.

3. Results

3.1. Morphology and distribution of cells in membrane

The ASCs in both processes are approximately φ20 μm after the digestion treatment by trypsin. After cell electrospinning, however, the cells in experimental membrane were fattened to about 35.4–44.9 μm, as shown in Fig. 1(A). This indicated that the cells were embedded in PVA (a), they were forming a local widening of the fibers, they aligned longitudinally with the fiber axis, and that there were few cells in each fiber. Moreover, Fig. 1