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Modulating the release of vascular endothelial growth factor by negative-voltage emulsion electrospinning for improved vascular regeneration

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ABSTRACT

Electrospun nanofibrous scaffolds providing local delivery of vascular endothelial growth factor (VEGF) have distinctive advantages for vascular tissue engineering. However, more than 90% of VEGF were normally released from scaffolds formed by conventional positive-voltage emulsion electrospinning (PVEES) within the initial 3 days. VEGF molecules bear positive charge. In this investigation, emulsion electrospinning using power supplies of different polarities was studied for producing scaffolds bearing specific electric charge. VEGF-containing poly(lactic-co-glycolic acid) scaffolds with initial potential of −87 and −202 V were formed by negative-voltage emulsion electrospinning (NVEES) at −10 and −20 kV, respectively, which enabled steady and sustained release up to 18 days, exhibiting effective modulation for VEGF release. Compared to VEGF-containing scaffolds formed by PVEES, NVEES-formed scaffolds showed superior performance in promoting endothelial cell functions.

1. Introduction

Electrospun scaffolds have great potential in vascular tissue engineering owing to their biomimetic nanofibrous architecture [1]. Incorporating vascular endothelial growth factor (VEGF) in scaffolds can enhance biological performance [2]. Emulsion electrospinning is an effective technique to make growth factor (GF)-containing scaffolds, where GFs are encapsulated and protected in the core of core-shell structured nanofibers [3]. However, conventional positive-voltage emulsion electrospinning (PVEES) has drawbacks as GF normally shows initial rapid release, which should be minimized. Obtaining scaffolds with steady and sustained release of GFs is important.

The GF release behavior from electrospun scaffolds could be modulated through electrostatic interaction by using a polyelectrolyte bearing electric charge [4]. However, this approach could undesirably change scaffold architecture from nanofibrous to microfibrous. Developing new strategies for modulating GF release from nanofibrous scaffolds is therefore needed. Most electrospun polymer fibers retain electric charges for certain time [5]. In this investigation, either PVEES or novel negative-voltage emulsion electrospinning (NVEES) was conducted, aiming to produce VEGF-encapsulated scaffolds carrying different types of electric charge. The scaffolds were characterized using different techniques. VEGF encapsulation efficiency and release behaviors were analyzed. Human vein umbilical endothelial cell (HUVEC) was cultured on VEGF-encapsulated scaffolds formed by PVEES or NVEES and behaviors of HUVEC cells on scaffolds were investigated.

2. Experimental

Poly(lactic-co-glycolic acid) (PLGA, LA:GA = 50:50, Mw = 120,000; Lakeshore Biomaterials Inc., USA) was used for making scaffolds due to its suitability for vascular tissue engineering [6]. Recombinant human VEGF (Mw = 40 kDa) was from PeproTech, USA. Chemicals and reagents for preparing emulsions were products of Sigma-Aldrich, USA. Primary HUVEC cells and Medium 200 with supplemented 2% v/v low serum growth supplements (M200) were from Life Technologies, USA. HUVEC cells were cultured in M200 in a 37 °C, 5% CO_2, humidified incubator. After 80% confluency, cells were trypsinized and counted using a haemocytometer for cell culture experiments.

Water-in-oil emulsions were prepared [3]. Briefly, PLGA was dissolved in dichloromethane at a PLGA concentration of 15% w/v as the "oil phase" and phosphate buffered saline with dissolved VEGF (10 μg/mL) and supplemented bovine serum albumin (BSA, 10 μg/mL, for stabilizing VEGF) was used as the "water phase".

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Stable emulsions were made at the water phase to oil phase volume ratio of 1:10 with a small addition of surfactant span 80. They were electrospun using PVEES or NVEES at different applied voltages (+10, +20, −10 or −20 kV) (Fig. 1a). The scaffolds formed were examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Surface potentials of scaffolds after electrospinning were measured [5] for up to 21 days using an electrostatic voltmeter (IsoProbe, Model 244A, USA). *In vitro* release tests were performed for VEGF-containing scaffolds using VEGF enzyme-linked immunosorbent assay (ELISA) kit (PeproTech, USA) [4]. Scaffolds made at +20 kV or −20 kV were sterilized by gamma-ray radiation and seeded with HUVEC cells (1×10^4 cells/well) in 48-well plates and cultured in a 37 °C, 5% CO_2, humidified incubator. After 3-day and 7-day cell culture, actin filaments (F-actin) and nuclei of HUVECs on scaffolds were respectively stained green by Alexa Fluor 488-conjugated phalloidin (Invitrogen, UK) and blue by 4-6-diamidino-2-phenylindole (DAPI; Invitrogen, UK). They were then visualized using fluorescence microscopy. Statistical analysis of results was performed.

3. Results and discussion

Through measuring the surface potential (n = 6; as indicator of electric charge) of VEGF-incorporated PLGA scaffolds made by PVEES or NVEES, it was revealed that scaffolds retained different electric charges, i.e., positive charge in PVEES-formed scaffolds and negative charge in NVEES-formed scaffolds (Fig. 1b). Regardless of PVEES or NVEES, scaffolds formed at higher voltages exhibited larger absolute values of charge (Fig. 1b). It was interesting that scaffolds formed by PVEES or NVEES at a voltage of the same

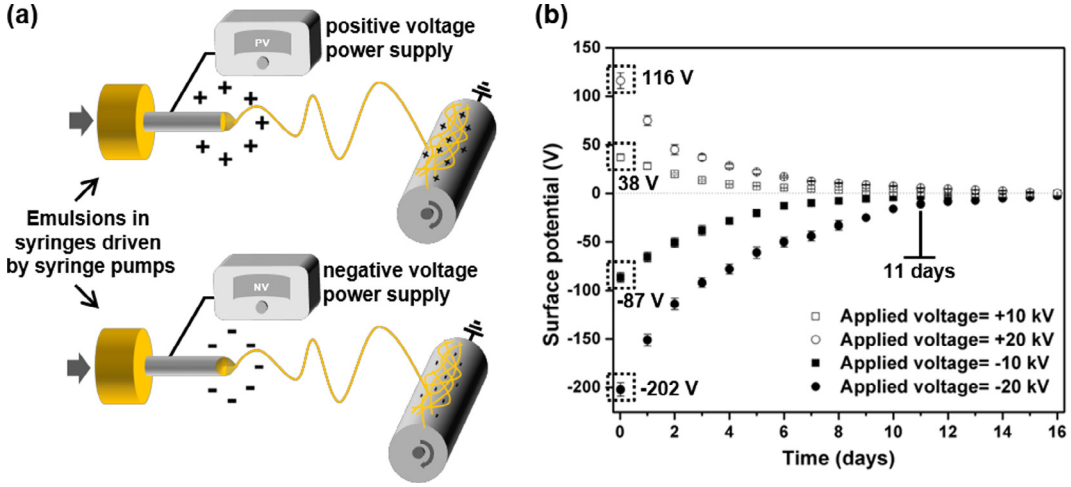

Fig. 1. (a) Schematics for PVEES (top) and NVEES (bottom), (b) Surface charge retention behaviors of scaffolds formed by NVEES or PVEES.

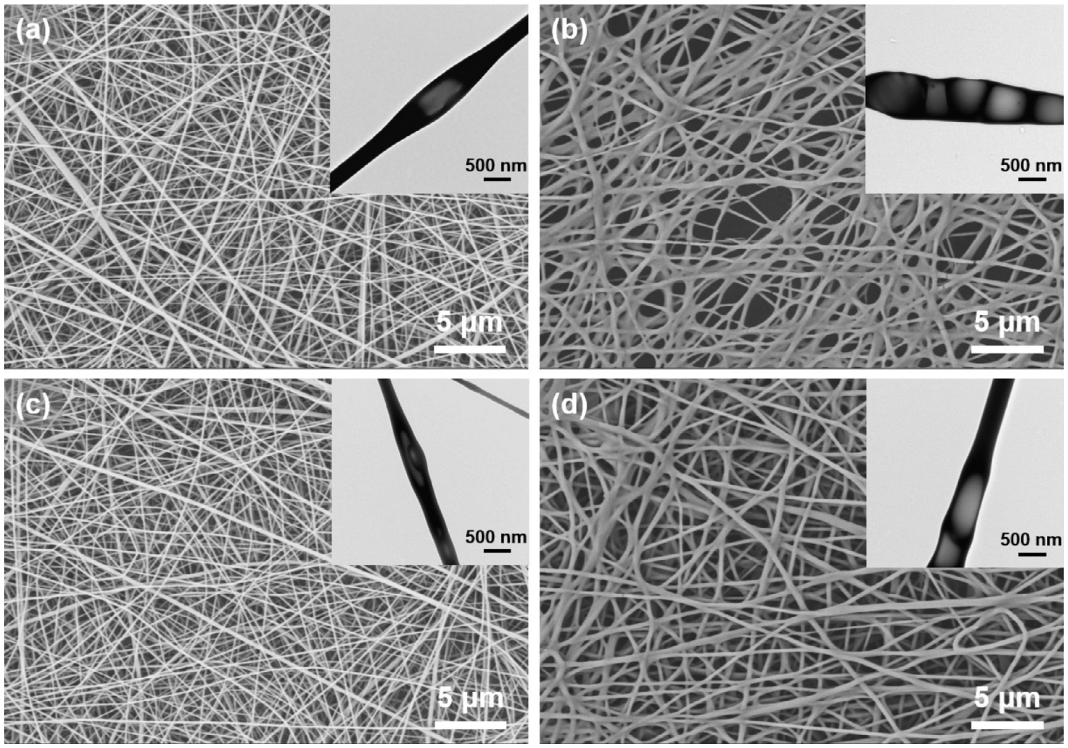

Fig. 2. Morphology and fiber structure (insets) of scaffolds formed by NVEES or PVEES: (a) PVEES, 10 kV, (b) PVEES, 20 kV, (c) NVEES, −10 kV, (d) NVEES, −20 kV.