Fabrication of Nanofiber Microspheres with Tunable Morphology via Gas Bubble-Mediated Co-axial Electrospray

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Statement of Purpose: Minimally invasive therapies avoiding surgical complexities evoke great interest in developing injectable biomedical materials.[1-3] Herein, we report a versatile approach for engineering injectable and biomimetic nanofiber microspheres (NMs) with tunable sizes, predesigned structures, and desired compositions via gas bubble-mediated co-axial electrospraying. The sizes and structures of NMs were controlled by adjusting processing parameters including air flow rate, applied voltage, distance, and spinneret configuration in the co-axial setup. Importantly, unlike the self-assembly method, this technique can be used to fabricate NMs from any material feasible for electrospinning or other nanofiber fabrication techniques.[4]

Methods: All the NMs in this study were fabricated by co-axial electrospraying of aqueous short nanofiber dispersions in the dripping mode. A piece of aluminum (Al) foil was immersed in the liquid nitrogen as the ground collector. Initially, the nanofiber mat of both PCL:gelatin was segmented by Cryostat cutting, freeze-dried, and kept at 4ºC for further use. The short nanofiber fibers were typically dispersed in water at a concentration of 20 mg/mL and gelatin (5%, with respect to the fiber content) was homogenized for 40 minutes. Then, the well-homogenized nanofiber-dispersed solution was used for the fabrication of various NMs by co-axial electro spraying techniques and followed by freeze drying and crosslinking at glutaraldehyde vapors to collect stable NMs.

Results: We examined a variety of parameters like flow rate of the air, distance between the spinneret and collector and voltage to tune the morphology and porosity of NMs. **Figure 1** shows SEM images of various PCL/gelatin NMs at airflow rates ranging from 1 to 10 mL/h. When applying the airflow rate at 1 mL/h, hollow NMs were obtained (**Figure 1**a,a_1,a_2). After increasing the airflow rate to 2 mL/h and 5 mL/h, the hollow NMs became partially porous (**Figure 1**b,b_1,b_2 and **Figure 1**c,c_1,c_2). After further increasing the airflow rate to 10 mL/h, completely open porous NMs were achieved (**Figure 2**d,d_1,d_2). The difference in morphologies could be due to the number of gas bubbles introduced within the droplets. At low airflow rates, bubbles were able to fuse and form a large bubble in the droplets with a pressure too low to break the nanofiber shell before freezing in the liquid N_2. Similarly, we fabricated different NMs as a function of distance, voltage alignment of coaxial system to tune the morphology of NMs.

To demonstrate cell delivery capabilities, human neural stem/progenitor cells (hNSCs) were seeded on both porous and nonporous PCL:gelatin NMs. The cell density on the nonporous NMs was lower compared to porous NMs. The interconnected pores in porous NMs provided more space for cell growth and enabled oxygen and nutrient diffusion, eventually forming 3D tissue spheroids. The injectable hNSC-seeded porous NMs at different levels of neuronal differentiation results shows great potential for treating neurodegenerative diseases and traumatic brain injuries. The H&E staining images of subcutaneously implanted porous NMs showed many host cells infiltrated and migrated throughout the porous NMs after implantation for 1 week, while cell infiltration was less visible in nonporous NMs. After 2 weeks, cells completely penetrated throughout the porous NMs, with only superficial penetration on nonporous NMs.

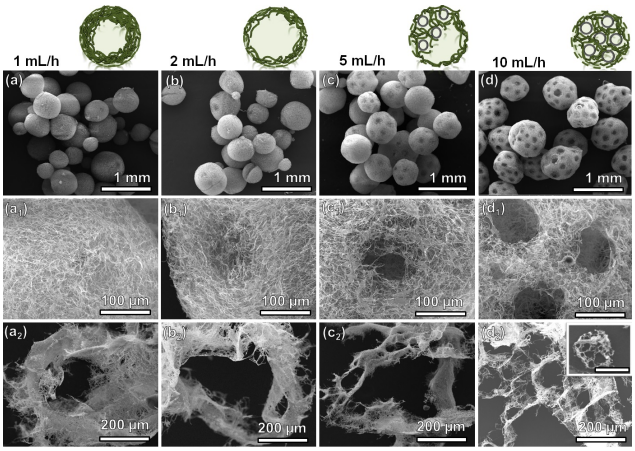

Figure 1. The effect of air flow rates on the formation of PCL:gelatin (1:1) NMs. (a-a_2) 1 mL/h. (b-b_2) 2 mL/h. (c-c_2) 5 mL/h. (d-d_2) 10 mL/h. The other processing parameters: short nanofiber concentration = 20 mg/mL, solution flow rate = 2 mL/h, applied voltage = 6 kV, distance between nozzle and collector = 10 cm.

Conclusions: The first time demonstrated a simple and versatile approach for engineering NMs with controlled size, predesigned structure, and desired composition by co-axial electrospraying of air in the core and a short nanofiber solution in the shell, respectively. Varying parameters, such as airflow rates and applied voltages, enables a tunability that is yet to be reported in engineering NMs. We also demonstrated that NMs serving as cell carriers can enhance expansion and differentiation, suggesting the potential use for stem cell therapies. The in vitro and in vivo studies showed that the open porous architecture of the NMs provided an ideal matrix for cellular infiltration and integration with host tissue.

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