Nanofibrous Scaffold Design for the Tympanic Membrane

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Introduction

Hearing loss resulting from tympanic membrane (TM) perforations is common across all ages. TM perforations that occur as a result of the disease process in chronic otitis media affect at least 0.5% population. Tympanoplasty can be quite successful in restoring the anatomic integrity of the TM and protecting the middle-ear cleft from opportunistic infection. However, closure of TM perforations with standard grafting materials, such as temporalis fascia or perichondrium, does not restore the complex microanatomy in the normal TM, i.e. the organization of collagen fibers in the TM. Clinical audiometers typically do not measure frequencies above 6 to 8 kHz, while normal ear can hear between 20 Hz to approximately 20 kHz. It is therefore highly desirable to develop fibrous scaffolds with structure similar to that of TM which can be implanted into the eardrum to guide TM tissue regeneration.

In our study, fiber arrangement in the rat TM was observed under high resolution scanning electron microscope (SEM). Aligned solid Poly(caprolactone) (PCL) fiber network was fabricated using electrospinning to mimic the fiber structure in the TM. Core-shell fibers were also electrospun to demonstrate the possibility of loading the scaffolds with active agents, such as growth factors, to promote cell attachment, migration and proliferation in the tissue regeneration process.

Materials and Methods

The TM specimens obtained from healthy adult rats were treated in 1% trypsin dissolved in phosphate buffer solution at 37°C and fixed in 2.5% glutaraldehyde solution. After dehydration in a series of ascending concentrations of aqueous ethanol and critical point dried, the specimens were coated with 3 nm osmium and observed in SEM.

PCL fibers were electrospun using 8 wt% PCL dissolved in trifluoroethanol. A rotating mandrel was used as a collector to control fiber orientation. Coaxial electrospinning was performed by feeding two solutions into a coaxial composite spinneret which consists of two concentrically arranged capillaries. Two types of core-shell fibers were prepared to confirm the core-shell structure. In one of them, the core solution was made by dissolving 100 mg of Bovine Serum Albumin (BSA)-Alexa Fluor® 594 in 10 mL poly(ethylene glycol) (PEG) solution which was composed of 200 mg/mL PEG in 80%~90% aqueous ethanol, and the shell solution was prepared by adding 84.2 mg of type I collagen from rat tail to 1 mL hexafluoroisopropanol (HFIP), while in the other the core solution was made by dissolving 100 mg of Bovine Serum Albumin-Fluorescin Isothiocyanate (BSA-FITC) in 10 mL 16.7% PEG in distilled water, and the shell solution was 8 wt% PCL.

Results

The mammalian TM pars tensa, which is responsible for transmitting acoustic vibration into

the middle ear, consists of three layers: outer epidermal, middle lamina propria, and inner mucous layer. The middle lamina propria is mainly formed of outer radial and inner circular fibers arranged in an orderly way. The radial fibers converge on the manubrium while the circular fibers become denser towards the periphery. Figures 1a and 1b show the radial and circular fibers observed from the external ear canal and middle ear cavity, respectively. In most area across the pars tensa, radial and circular fibers are perpendicular to each other (Fig. 1c).

Two layers of fibers perpendicular to each other (Fig. 2a) can be achieved by using a rotating mandrel as a collector in electrospinning. Figure 2b shows the TEM image of a BSA-Alexa Fluor® 594/Collagen core-shell fiber, which reveals a well-defined core-shell structure composed of darker region BSA-Alexa Fluor® 594 proteins and lighter region type I collagen. The confocal image (Fig. 2c) indicates the encapsulation of BSA-FITC in PCL shell, which demonstrates the possibility of loading fibrous scaffold for drug delivery and tissue engineering applications.

Discussion

It is well known that the arrangement of collagen fibers plays an important role in the mechanical function of the TM. O'Connor et al. reported that the radially aligned collagen fibers in human TM are crucial in the transmission of sound above 4 kHz. It is therefore important not only to restore the anatomic integrity of the TM in tympanoplasty, but also to restore the complex microanatomy in the normal TM. Our experiments demonstrate the possibility of fabricating a fibrous scaffold consisting of core-shell fibers with structure similar to the TM fiber network, which might be able to be applied to a TM perforation and guide the TM tissue regeneration.

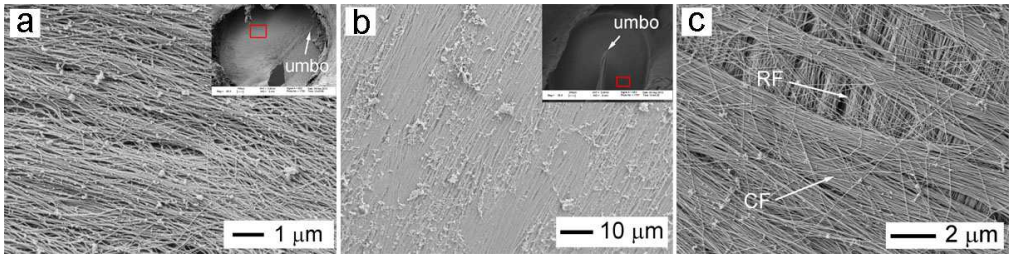

Figure 1: SEM images of radial fibers (a) observed from external ear canal, and circular fibers (b) observed from middle ear cavity with the red rectangle in the insets showing the relative positions of the images in the TM; (c) radial and circular fibers observed from middle ear cavity at a location where there are clefts between radial fibers.

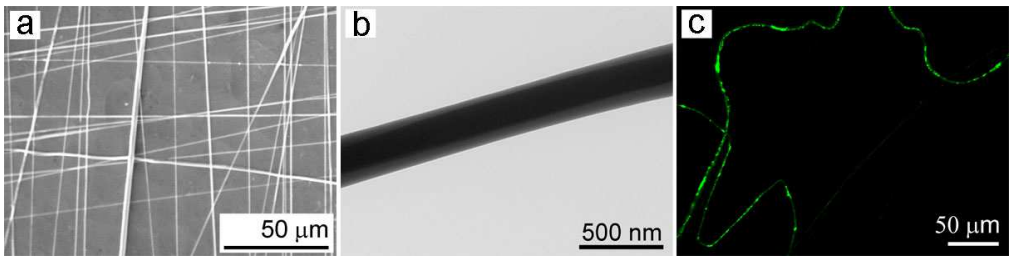

Figure 2: (a) SEM image of two layers of PCL fibers aligned perpendicularly. (b) TEM image of a BSA-Alexa Fluor® 594/Collagen core-shell fiber. (c) Confocal image of BSA-FITC/PCL core-shell fibers.