Materials and Design 122 (2017) 206–219

Contents lists available at ScienceDirect

Materials and Design

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

Design, fabrication and characterization of composite piezoelectric ultrafine fibers for cochlear stimulation

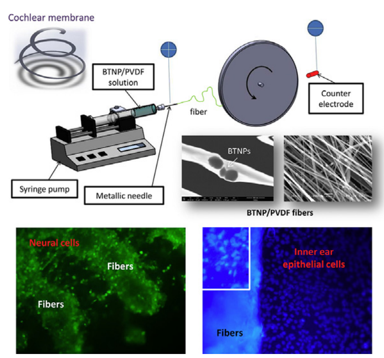
Carlos Mota [a,b], Massimiliano Labardi [c], Luisa Trombi [a], Laura Astolfi [d], Mario D'Acunto [e], Dario Puppi [b], Giuseppe Gallone [f], Federica Chiellini [b], Stefano Berrettini [a,g], Luca Bruschini [a,g], Serena Danti [a,f,*]

[a] OtoLab, Otorhinolaryngology, Audiology & Phoniatrics Unit, Azienda Ospedaliero-Universitaria Pisana (AOUP), Pisa, Italy
[b] BIOLab, Dept. of Chemistry and Industrial Chemistry, UdR INSTM Pisa, University of Pisa, Pisa, Italy
[c] Institute for Chemical-Physical Processes, National Research Council (CNR-IPCF), Pisa, Italy
[d] Bioacoustics Research Laboratory, Dept. of Neurosciences, University of Padua, Padua, Italy
[e] Institute of Science of Matter, National Research Council (CNR-ISM), Rome, Italy
[f] Dept. of Civil and Industrial Engineering, University of Pisa, Pisa, Italy
[g] Dept. of Surgical, Medical, Molecular Pathology and Emergency Medicine, University of Pisa, Pisa, Italy

HIGHLIGHTS

- A method was designed to increase sensitivity and performance of piezoelectrodes for cochlear stimulation.
- Ceramic/polymer composites were used to increase piezoelectric properties, anisotropic fibers to enhance the neurite/material interaction.
- Fiber alignment increased with tangential velocity of the collector, but fiber diameter was not affected.
- The piezoelectric coefficients proportionally increased with barium titanate weight percentage in the composites.
- The fibers were cytompatible and, under dynamic culture, enhanced the viability of neural cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 November 2016
Received in revised form 3 March 2017
Accepted 4 March 2017
Available online 06 March 2017

Keywords:
Electrospinning
Barium titanate
Polyvinylidene fluoride
Aligned fibers
Neural cells
Transducer

ABSTRACT

Sensorineural hearing loss, primed by dysfunction or death of hair cells in the cochlea, is the main cause of severe or profound deafness. Piezoelectric materials work similarly to hair cells, namely, as mechano-electrical transducers. Polyvinylidene fluoride (PVDF) films have demonstrated potential to replace the hair cell function, but the obtained piezoresponse was insufficient to stimulate effectively the auditory neurons. In this study, we reported on piezoelectric nanocomposites based on ultrafine PVDF fibers and barium titanate nanoparticles (BTNPs), as a strategy to improve the PVDF performance for this application. BTNP/PVDF fiber meshes were produced via rotating-disk electrospinning, up to 20/80 weight composition. The BTNP/PVDF fibers showed diameters ranging in 0.160–1.325 μm. Increasing collector velocity to 3000 rpm improved fiber alignment. The piezoelectric β phase of PVDF was well expressed following fabrication and the piezoelectric coefficients increased according to the BTNP weight ratio. The BTNP/PVDF fibers were not cytotoxic towards cochlear epithelial cells. Neural-like cells adhered to the composite fibers and, upon mechanical stimulation, showed enhanced viability. Using BTNP filler for PVDF matrices, in the form of aligned ultrafine fibers, increased the piezoresponse

* Corresponding author at: Dept. of Civil and Industrial Engineering, Largo L. Lazzarino 2, 56122 Pisa, Italy.
E-mail address: serena.danti@unipi.it (S. Danti).

http://dx.doi.org/10.1016/j.matdes.2017.03.013

of PVDF transducers and favored neural cell contact. Piezoelectric nanostructured composites might find application in next generation cochlear implants.

1. Introduction

Sensorineural hearing loss (SNHL) is a major impairment of the inner ear as a consequence of damaged sensory epithelium. These cells, known as hair cells, are present in the organ of Corti to convert mechanical vibration of the basilar membrane into electric stimulation of the underlying neurons. Dysfunction of the mechano-electrical transduction capability of hair cells leads to deafness. Although ageing or external factors, such as exposure to noise and ototoxic effects of certain drugs, can affect the viability and function of the hair cells, in most cases (>60%) SNHL is associated to inherited genetic conditions [1–3]. To treat SNHL, several approaches, such as gene therapies, stem cell-based treatments, neuroprotective and chemical drugs, are being investigated aimed at the regeneration of the delicate inner ear microenvironment [3,4]. Despite of these new biological and pharmaceutical strategies, nowadays, the only successful treatment for SNHL still relies on the use of cochlear implants (CIs), which involve an invasive and complex surgery of the temporal bone [2]. The CI is a multi-component electronic device that completely replaces the ear function. It includes an external receiver powered by a battery in which the sound is captured and processed and finally transmitted to a subcutaneous receiver that is connected to the electrode array implanted inside the cochlea [5]. The electrode array directly stimulates the auditory nerves, bypassing the need for hair cells as transducers. Since the fifties, different electrode arrays have been developed, composed with a variable number of electrodes and configurations; however, so far, tone recognition of speech or music remains the pinnacle of CI-implanted patients [6,7].

Piezoelectric materials have recently been explored in diverse fields, including biomedicine and energy harvesting [8,9]. The development of novel CIs evolving towards the utilization of piezoelectric materials was firstly suggested by Mukherjee et al. at the beginning of this century [10–12]. Unlike conventional CIs, piezoelectric CIs are not ferromagnetic and exploit the cochlear constitutive mechanical tonotopy (i.e. frequency selectivity along the main axis of the basilar membrane), enabling a fine tuning process of the sound vibrations [13]. Frequency selectivity, travelling waves and tonotopic organization in an artificial membrane have been demonstrated [14]. Indeed, according to the travelling wave theory, tuning for sound frequency is largely determined by position. A piezoelectric polymeric film made of polyvinylidene fluoride (PVDF) was able to work as a stand-alone device, namely, without the necessity of an external power supply, thus resulting extremely appealing to reproduce an artificial cochlea [14]. For the fabrication of cochlear microdevices that must be flexible to accommodate the curvy inner ear anatomy, PVDF is indeed an interesting material, as it owns good piezoelectric properties together with the excellent processability proper of polymers [15,16]. The development of PVDF-based film membranes for cochlear stimulation has become a subject of few recent studies, showing the potential of changing the future way of treating SNHL, even though an insufficient sensitivity has been reached so far using plain PVDF [11,14,17].

On the other hand, ceramic materials with a perovskite-like structure, possess higher piezoelectric properties than those of polymers, but are very rigid and difficult to process. Among them, barium titanate ($BaTiO_3$) is a ceramic with excellent piezoelectric properties routinely applied in devices such as piezoelectric actuators and capacitors [18]. In recent years, barium titanate has also become a subject of biological and biomedical studies [19]. First evidences have shown that $BaTiO_3$ nanoparticles (BTNPs) were biocompatible and could be uptaken by cells with neither inducing apoptosis nor affecting cell metabolism and viability. BTNPs were also used as a filler for polymeric-matrix biomedical composite and as a carrier for an anticancer drug, thus appearing a versatile tool in biomedical applications [20]. It has also been demonstrated that BTNPs increased the piezoelectric properties of films made of a PVDF family copolymer [21].

To maximize the efficiency of a piezoelectric substrate towards the resident neural cells, an effective nanoscale contact between the neurites and the material must occur, which sometimes is difficult to achieve by using non-porous flat substrates, like films. Uniaxially aligned fibrous materials have shown topographic features providing favorable interactions with neurons and showing the ability to direct neurites [22]. Electrospinning is a widely used technique to manufacture fibrous materials for variety of applications, such as nanofiber-reinforced composites, membranes, smart cloths, electrode materials, sensors, as well as electronic and optical devices [23–25]. In the biomedical field, this techniques has gained a large popularity for its simplicity, cost-effectiveness and ability to produce nano-sized fibers resembling those of the native extracellular matrix, thus showing architectural cues suitable for cell interaction [26,27]. Electrospun fibers can be produced with diameters ranging from a few micrometers down to tens of nanometers, leading to meshes that present superior surface area-to-volume ratios. Specifically, electrospinning offers several theoretical advantages for producing piezoelectric substrates for cochlear stimulation: (*i*) it allows the fabrication of ceramic/polymer nanocomposites by incorporating nanoparticles in the polymeric solution, (*ii*) it inherently induces a poling effect on the piezoelectric fibers during the spinning process by means of the high electric field necessary for manufacturing, and finally (*iii*) it permits fiber deposition geometry to be tuned, which can be of outmost importance to induce specific cellular signals.

The aim of this study was the production of aligned ultrafine PVDF/BTNP piezoelectric fiber meshes via electrospinning with increased piezoelectric properties with respect to the plain PVDF and with morphological nano/micro features attractive for neural cells, as a step forward towards the accomplishment of piezoelectric substrates for CIs. Fiber morphology and alignment were evaluated via scanning electron microscopy (SEM) and two-dimensional Fast Fourier Transform (FFT), whereas single fiber topography was investigated via atomic force microscopy (AFM) and scanning near optical microscopy (SNOM). The dispersion of BTNPs inside the composite fibers was investigated using scanning transmission electron microscopy (STEM). The crystallographic and compositional characterization of the composite was performed via X-ray diffraction (XRD) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The mechanical behavior of the composite was tested under in situ environmental SEM (ESEM) tensile deformation and dynamic-mechanic thermal analysis (DMTA). Piezoelectric coefficients were evaluated as a function of BTNP concentration in the polymer using a custom-made bench apparatus. Finally, the composite material was cultured in vitro with inner ear epithelial cells to assess its cytocompatibility, and with neural-like cells using a bioreactor capable of imparting cyclic bending solicitation during cell culture, as a preliminary test to support its possible application in CI.

Advancements in the development of high performance piezoelectric biomaterials to be used as cochlear transducers would allow SNHL treatment to move forward the next generation CIs. Changing from electronics-based to biomaterials-based CIs, the latter being cost-effective, simple, biomimetic, water and magneto-compatible, would drastically improve the quality of life of deaf people.