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Developing robust, hydrogel-based, nanofiber-enabled encapsulation devices (NEEDs) for cell therapies

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ABSTRACT

Cell encapsulation holds enormous potential to treat a number of hormone deficient diseases and endocrine disorders. We report a simple and universal approach to fabricate robust, hydrogel-based, nanofiber-enabled encapsulation devices (NEEDs) with macroscopic dimensions. In this design, we take advantage of the well-known capillary action that holds wetting liquid in porous media. By impregnating the highly porous electrospun nanofiber membranes of pre-made tubular or planar devices with hydrogel precursor solutions and subsequent crosslinking, we obtained various nanofiber-enabled hydrogel devices. This approach is broadly applicable and does not alter the water content or the intrinsic chemistry of the hydrogels. The devices retained the properties of both the hydrogel (e.g. the biocompatibility) and the nanofibers (e.g. the mechanical robustness). The facile mass transfer was confirmed by encapsulation and culture of different types of cells. Additional compartmentalization of the devices enabled paracrine cell co-cultures in single implantable devices. Lastly, we provided a proof-of-concept study on potential therapeutic applications of the devices by encapsulating and delivering rat pancreatic islets into chemically-induced diabetic mice. The diabetes was corrected for the duration of the experiment (8 weeks) before the implants were retrieved. The retrieved devices showed minimal fibrosis and as expected, live and functional islets were observed within the devices. This study suggests that the design concept of NEEDs may potentially help to overcome some of the challenges in the cell encapsulation field and therefore contribute to the development of cell therapies in future.

1. Introduction

Cell encapsulation is a technology with enormous clinical potential for the treatment of a wide range of difficult diseases including type 1 diabetes [1–3], neurological and sensory diseases [4], cancers [5], and many others [6–8]. In cell encapsulation, a biomaterial or device with semipermeable membranes protects the transplanted therapeutic cells from immune rejection, without the use of immunosuppression, while simultaneously allowing facile mass transfer to maintain the cell survival and function [9,10]. Polymer-based cell encapsulation devices have been developed for decades with some of them already commercialized, such as TheraCyte™ [11,12]. Although mechanically durable and easy to use, these current encapsulation devices, mostly made from porous membranes such as phase-inverted poly(acrylonitrile-co-vinyl chloride) [13,14] or expanded Teflon [11], have potential issues with fibrosis and insufficient biocompatibility [2,3].

As an alternative, hydrogels have been extensively investigated for cell encapsulation and delivery [15–20]. Among them, alginate hydrogel is one of the most common ones due to its easy and mild gelation as well as its relative biocompatibility [20–22]. In fact, alginate hydrogels in the form of microcapsules [23,24] have been used for decades for cell encapsulations especially as a potential treatment for type 1 diabetes. The microcapsules are easy to implant and have large surface areas for mass transfer, and tremendous promising results have been reported [25–27]. However, one concern is that after these microcapsules are implanted, often in the peritoneal cavity with a large number (~100,000 for a human), it may be inconvenient and time consuming to completely retrieve or replace them in the event when the transplant fails or medical complications occur [3,28]. In addition, it is challenging to control the locations of the cells within the microcapsules, leading

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to incomplete encapsulation and inadequate immunoprotection [10,29,30]. Lastly, the microcapsules sometimes tend to clump over time in the body, deteriorating the mass transfer [31].

To this end, macroscopic hydrogel devices such as long fibers [32] and thin sheets [33] have recently been proposed as alternative formats for cell encapsulations. However, the intrinsic softness of hydrogels due to the large water content (>95%) and the high aspect ratios of these macrodevices make the mechanical durability a potential concern for long-term clinical uses. In fact, most hydrogels that are suitable for cellular engineering applications [18,19] (i.e. with large water contents and high diffusion rates) tend to have relatively low mechanical strength, as compared to typical plastics or rubbers [34,35]. Robust mechanical properties are particularly desirable for cell encapsulation because the devices are intended for long-term use in the body. Numerous approaches have been reported to improve the mechanical properties of hydrogels, most commonly through increase of crosslinking densities [36] or incorporation of additional chemically crosslinked networks [35,37,38]. Here, we report a physical approach to fabricate robust hydrogel-based, ***n***anofiber-***e***nabled ***e***ncapsulation ***d***evices (or NEEDs) for cell encapsulation. To make the NEEDs, we make use of the capillarity-driven wicking phenomenon and infiltrate the nanofibrous walls of pre-made electrospun tubes or chambers with hydrogel precursor solutions. The precursors are held in place by the capillary force and the NEEDs, either tubular or planer, are obtained by a subsequent hydrogel crosslinking. This approach is simple, broadly applicable and does not alter the water content or the intrinsic chemistry of the hydrogels.

The NEED design takes advantages of the mechanical strength and the unique, fine pore structures of the electrospun nanofiber membranes. Electrospun nanofibers are a versatile class of material that has various attractive properties for use as biomaterials [39,40] such as the small fiber size (~10 nm–10 μm), high porosity (>90%), large surface area (~10 m^2/g), and interconnected pore structures (~1 μm). Depending on the chemical compositions, they also have tunable material properties including mechanical strength, biodegradability and wettability [39–41]. It has been shown that by controlling how they are collected during the electrospinning process it is possible to engineer the nanofibers into macroscopic devices such as microwell chips [42] or microtubes [43,44]. Given these unique properties, electrospun nanofibers provide an enabling platform to engineer the next generation of hydrogel-based cell encapsulation devices.

The NEEDs have several advantages for cell encapsulation. First, the nanofiber membranes as the scaffolds of the device walls provide the necessary mechanical strength and prevent any potential breakage or cell leakage while still allowing adequate mass transfer. Second, the hydrogel as the device exterior reinforced by the nanofibers through mechanical interlocking provide the necessary biocompatibility and immunoprotection. Third, the NEEDs can be pre-fabricated and the cells can be loaded in a custom designed fashion, for example, by dispersing the cells in physiologically relevant extracellular matrices (ECM) [45]. This way, the device exterior hydrogel that interacts with the body when transplanted and the ECM hydrogel in the internal compartment that interacts with the cells can be decoupled and independently designed. Finally, multiple compartments can be engineered into a single NEED, which can then be used for complex cell encapsulation, co-culture and delivery.

In this work, we fabricated the NEEDs with different hydrogel chemistries and compartmentalizations. Through tensile tests, we confirmed their robust mechanical properties. Using model cells, we demonstrated the facile mass transfer and flexible cell loading in single or multiple compartments with a control over the cell-dispersing matrix. Lastly, we evaluated the potential application of the devices by encapsulating and delivering insulin-producing rat pancreatic islets into a chemically-induced diabetic mouse model. The diabetes was corrected for the duration of the experiment (8 weeks) before the implants were retrieved. The retrieved devices showed minimal fibrosis according to histological studies and as expected, live and functional islets were observed within the devices. This work provides a proof of concept for the NEEDs as a new platform for potential cell encapsulation therapies.

2. Materials and methods

2.1. Chemicals

Poly(caprolactam) (Nylon 6), polysulfone (PSU), polyacrylonitrile (PAN) and polycaprolactone (PCL) were purchased from Scientific Polymer Products, Inc. (Ontario, NY). Formic acid, N,N-dimethylformamide (DMF), dichloromethane (DCM), N,N-dimethylacetamide (DMAc), poly(ethylene glycol) diacrylate (PEG-DA), 2-hydroxy-2-methylpropiophenone, $CaCl_2$ and $BaCl_2$ were purchased from Sigma–Aldrich Co. (St. Louis, MO). Sodium alginate was purchased from FMC BioPolymer Co. (Philadelphia, PA). All reagents were purchased and used as received without further purification.

2.2. Animals

Immune-competent male C57BL/6 mice were obtained from Jackson Lab and Sprague–Dawley rats were obtained from Charles River Laboratories. All animal procedures were approved by the Cornell Institutional Animal Care and Use Committee.

2.3. Fabrication of electrospun nanofiber tubes or chambers

In a typical procedure for electrospinning, a solution of 20% (w/v) Nylon 6 in formic acid was used. The nanofibers were spun at 20 kV (Gamma High Voltage, Ormond Beach, FL) with a pumping rate of 0.001 mL/min (Harvard Apparatus, MA) and with a 16G 1″ blunt needle as the spinneret. Working distance was fixed at 25 cm. A rotating target (i.e. aluminum rods with diameters ranging from 0.32 mm to 2.41 mm or aluminum plates with various dimensions) was placed in the path of the polymer solution jet. The rod was connected to an AV motor controlled by rheostat (VWR) and rotated at 400–500 rpm. After electrospinning process, the nanofibrous tubes or chambers were removed from the template and cut into desired lengths, or placed in a hydrogel precursor solution to make NEEDs (see Section 2.4 below). For other polymer nanofibers, the solutions and electrospinning conditions are summarized in Table 1. To fabricate the multi-compartmental devices, several pre-made nanofiber tubes in the presence of the rod templates were bundled together and used to further collect electrospun nanofibers.

2.4. Fabrication of NEEDs

The as-prepared electrospun nanofiber device (tubular or planar, in the presence of the template) was submerged in a 2% (w/v) solution of SLG20 alginate dissolved in 0.8% (w/v) NaCl solution. The whole setup was put in a vacuum chamber for degasing for 15 min to ensure the full impregnation of the alginate solution into the interstitial space of the nanofiber membrane. The alginate was then crosslinked by submerging the device into a $BaCl_2$/mannitol/HEPES solution ($BaCl_2$: 20 mM, D-mannitol: 250 mM, KCl: 2 mM, HEPES: 10 mM). The NEEDs were washed with 1× PBS for 3 times and were ready to use. Devices based on chitosan and collagen hydrogels were similarly prepared. The chitosan was crosslinked with a triphosphate solution and the collagen by neutralizing the pH and incubation at 37 °C. For PEG hydrogel device, a PEG precursor solution composed of 2-hydroxy-2-methylpropiophenone:PEG-diacrylate (PEG-DA):water (0.5:50:50 wt:wt:wt) was prepared first. Then a nanofiber tube was submerged in the PEG-DA solution and degased in a vacuum chamber for 15 min. The device with impregnated PEG precursor was put in the UV crosslinker (Spectronics, XL-1000) and exposed to UV radiation (325 nm) for 300 mJ/cm^2.

Table 1
Electrospinning parameters for different polymer nanofiber devices.

Polymer	Solvent	Concentration (w/v)	Voltage (kV)	Working distance (cm)
Poly(caprolactam) (Nylon 6)	Formic acid	20%	20	25
Polyacrylonitrile (PAN)	DMF	7.5%	18	22
Polycaprolactone (PCL)	DCM	30%	10	30
Polysulfone (PSU)	DMAc	25%	19	27
Polystyrene (PS)	DMF	20%	16	30