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Fabrication of fibrous silica sponges by self-assembly electrospinning and their application in tissue engineering for three-dimensional tissue regeneration

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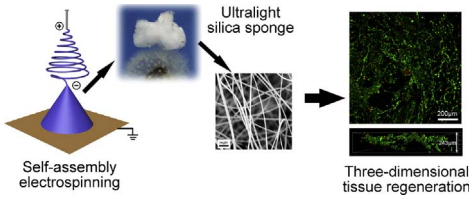
GRAPHICAL ABSTRACT



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ABSTRACT

Fabrication of highly porous, three-dimensional (3D) scaffolds with biomimicking microstructures for tissue engineering has received extensive attention lately. Although several studies have used silica as a filler material in various polymeric scaffold matrices for tissue engineering, there are no reports of 3D scaffolds fabricated solely with silica. In this study, we developed a method to fabricate fibrous silica sponges using a tetraethyl orthosilicate (TEOS)/polyvinyl alcohol (PVA) solution via self- assembly electrospinning and subsequent calcination. We then evaluated its potential application in tissue engineering. A detailed mechanism study revealed that appropriate crosslinking between hydrolyzed TEOS and PVA was the key to inducing 3D fiber stacking. The prepared silica sponges had a bulk density of 16 mg/cm^3, a surface area of 6.45 m^2/g, and a porosity of 98%, which endowed them with super-high absorbability. Cell culture results with 3T3 fibroblasts confirmed that the cells interacted strongly with the 3D silica fibers, showing a higher viability and proliferation rate than on 2D silica membranes, and that the cells migrated into the inner area of the sponge rapidly, indicating these silica sponges have potential to be used for 3D tissue regeneration.

1. Introduction

Electrospinning is an effective and economical technique for producing continuous fibers with a diameter range from hundreds of nanometers to tens of micrometers from a concentrated solution or melt under a strong electric field [1]. The electrospun fibers have distinct advantages, such as high aspect ratios, large surface area-to-volume ratios, open porous structures, and are lightweight. The morphology of electrospun fibers also resembles that of the native collagen fibrils in the extracellular matrix (ECM). As a result, electrospun fibers have found applications in various fields including biomedical, biotechnology, environmental engineering, electrodes, filtration,

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nanocatalysis, pharmaceutical, protective clothing, sensors, and tissue engineering scaffolds [2–6]. Even though electrospinning is versatile and has extensive applications, a significant drawback is that it has a highly compact two-dimensional (2D) membrane geometry that limits its application in three dimensions. Theoretically, fibrous sponges with a 3D structure would have much higher surface area-to-volume ratios, lower apparent densities, better filtration properties, superior absorption capacities, and better suitability to support cell growth in three dimensions as they do *in vivo* compared to 2D fibrous membranes.

Several methods have been developed to prepare 3D fibrous sponges via electrospinning. Simply increasing the electrospinning time to several hours only increases the thickness of the fiber mats to hundreds of microns. Using a 3D collecting template or dynamic collecting device instead of a flat aluminum foil collector, three-dimensional random fibers or woven fibers can be successfully obtained [7,8]. Layer-by-layer stacking and compression is a simple post-processing method to build up thickness but it lacks continuity [9]. Dispersion and freeze drying of electrospun mats is another post- processing method that retains the high porosity and fibrous structure, but the fiber length is seriously reduced because of the high speed of homogenization [10,11]. Using chemical blowing agents [12], liquid-assisted collection methods [13,14], adding additional electric or magnetic fields [15], using solutions with opposing charges [16], or using high humidity conditions [17] are other alternative methods for fabricating 3D fibers using electrospinning. Apart from these methods, the self- assembly technique is the simplest and most cost-effective method since it does not require any additional setup, steps, or environmental modifications [18]. However, it is also the hardest to achieve because the electrospinning solution is the only factor that can be adjusted given that the other electrospinning parameters do not have a significant effect on the formation of 3D fibers.

So far, several neat materials and hybrid materials have been discovered or developed to induce the self-assembly of 3D fibers. Polystyrene (PS) was found to be the most common polymer capable of being directly electrospun into 3D stacks [19]. It can also be combined with other polymers to form 3D fiber stacks, including polyacrylonitrile (PAN) [20], poly(ε-caprolactone) (PCL) [21], and PCL/$FeCl_3$ composites [22]. Bonino et al. successfully prepared 3D polyethylene oxide (PEO) fibers by combining them with negatively charged alginate in a controlled humidity environment [23]. Metallic nitrates have been added into polymers such as polyvinylpyrrolidone (PVP) [24] and PS [19] to increase the evaporation of the solvent and the solidification speed of the polymer to achieve 3D stacked fibers. These are the only materials that have been successfully used to fabricate 3D electrospun fibers in a self-assembled fashion, and they are all polymer-based materials which have limited usage in high- temperature, corrosive, and other harsh environments.

Inorganic ceramics have excellent thermal resistance, high rigidity, wear resistance, and oxidative and chemical resistance, which make them suitable in many critical conditions. Silicon dioxide (silica) is a readily available and low cost material that has been extensively used. Silica nanoparticles have also been extensively used in bone tissue engineering and are able to induce bone regeneration [25]. So far, the majority of studies only used silica as a filler material in a polymeric matrix such as PCL [26], chitosan [27], gelatin [28], and poly(lactic-co-glycolic acid) (PLGA) [29]. There have been no reports of scaffolds fabricated solely with silica, which might be due to the brittleness of silica and the limited 2D geometries. When fabricated into fibers, bulk silica fibrous material possess robust properties. It has been used in broad applications including catalysis [30], adsorption [31], sensors [32], encapsulation [33], and integrated circuits [34]. To date, producing silica fibers with diameters in the submicron and nanometer scale, as well as three-dimensional structures, is still challenging. Silica fiber mats have been prepared via sol-gel electrospinning using TEOS [35]. Various polymer carriers have been used in the preparation of silica or silica/polymer hybrid electrospun fibers, and the effects of various parameters have also been investigated [34,36–39]. However, there has not been a report of the fabrication of 3D silica fibers, and their potential applications in tissue engineering have not been investigated.

In this study, we found that electrospun fibers could self-assemble into 3D stacks using specific condensed TEOS/polyvinyl alcohol (PVA) solutions under mild electrospinning conditions; namely, room temperature and humidity. Subsequently, 3D silica sponges could be easily obtained by calcination. The produced silica sponges have the following characteristics: ultra-light weight, high surface areas and porosity, and excellent absorption capabilities compared to 2D silica membranes and commercial cotton. In terms of biocompatibility and potential tissue engineering applications, 3T3 fibroblast cells on the silica sponges showed high viability and proliferation rates, and favorable cell–substrate interactions. More importantly, cells were able to easily penetrate into the 3D fibers.

2. Experimental methods

2.1. Materials

Reagent-grade tetraethyl orthosilicate (TEOS), poly(vinyl alcohol) (PVA) (M_w = 89,000–98,000), and phosphoric acid (H_3PO_4) solution (85 wt% in H_2O) were purchased from Sigma–Aldrich without further purification. PVA was pre-dried in a vacuum oven at 75 °C for 1 h to remove moisture. Deionized water was used throughout the experiment. All reagents used for biological experiments were purchased from ThermoFisher Scientific unless otherwise specified.

2.2. TEOS/PVA solution preparation

The silica precursor solution was prepared by adding TEOS (11.26 mL) into DI water (10 mL) followed by the dropwise addition of H_3PO_4 (63 μL). The solution was stirred at room temperature for 10 h to form a hydrolyzed homogenous solution. The PVA solution was prepared by dissolving PVA (1 g, 1.4 g, and 1.8 g) into DI water (10 mL) at 80 °C for 4 h. The two solutions were mixed and stirred for 3 h at room temperature. Then the mixed solution was heated at 60 °C for different periods of time and used for electrospinning.

2.3. Electrospinning and calcination

The prepared solutions were loaded into a polypropylene syringe connected to an 18-gauge blunt-end needle and then mounted on a digital syringe pump (Harvard Bioscience Company). The electrospinning procedure was carried out using an 18 kV voltage, a 200 mm needle-to-target distance, and a 1 mL/h flow rate for 120 min for all solutions. The fibers were directly deposited on a piece of grounded aluminum foil. The obtained TEOS/PVA as-spun fibers were heated to 500 °C with a 10 °C/min heating rate and held for 2 h, then heated to 800 °C with a 10 °C/min heating rate and held for 3 h in air using a furnace (ThermoScientific) to obtain the silica fibers. In this process, both 3D sponges and 2D membranes were obtained depending on the solution's properties. The 3D sponges were fabricated using a 14% PVA solution and an aging time of 3 h. For comparison, 2D membranes were fabricated using a 14% PVA solution without aging.

2.4. 3T3 fibroblast cell culture

NIH 3T3 fibroblasts were treated with ethylenediaminetetraacetic acid (EDTA) for 5 min and then washed with phosphate-buffered saline (PBS) prior to seeding. Silica sponges, silica membranes, and coverslips as a control were sterilized using an autoclave. They were seeded with cells at a seeding density of 3×10^5 cells/cm^2 for cell attachment, 1.25×10^5 cells/cm^2 for live/dead and MTS assays, and 5×10^4 cells/cm^2 for cytoskeleton studies. Cells were fed regularly with a 20% fetal