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# Multi-layered macroporous three-dimensional nanofibrous scaffold via a novel gas foaming technique

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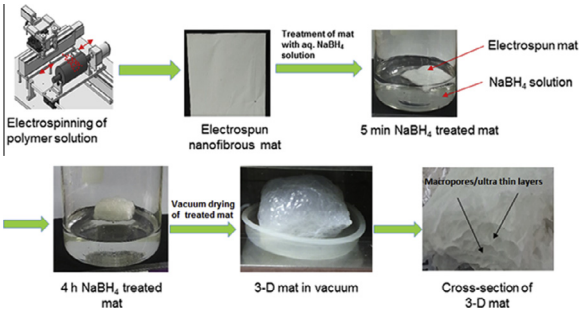
## HIGHLIGHTS

- Gas foaming technique is applied to prepare a 3-D nanofibrous scaffold.
- Sodium borohydride solution is introduced as gas foaming reagent.
- Novel mechanism for *in situ* gas foaming is demonstrated.
- Nature of the polymers affects the fabrication process.

## GRAPHICAL ABSTRACT

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## ABSTRACT

In the past decade, considerable efforts have been made to fabricate the biomimetic scaffolds from electrospun nanofibers for tissue engineering applications. However, one of the major concerns with electrospun nanofibrous scaffolds is the densely packed fibers in two-dimensional (2-D) array which impedes their applicability in tissue regeneration. To overcome this problem, a simple and facile post-electrospinning procedure was developed to modify a densely packed 2-D electrospun membrane into low density three-dimensional (3-D) scaffolds. In this strategy, an electrospun nanofibrous mat was immersed in a sodium borohydride (SB) solution. The interconnected pores of a mat are filled with the SB solution driven by capillary forces where it undergoes hydrolysis to produce hydrogen gas. The *in situ* generated gas molecules form clusters to minimize the free energy resulting in pore nucleation that reorganizes the nanofibers to form a low density, macroporous, spongy and multi-layered 3-D scaffold. Electrospun mats of various polar and non-polar polymers were subjected to post-electrospinning process to monitor the fabrication process. It has been found that the solvent for sodium borohydride (either water or methanol) played a crucial role in post-electrospinning process. Only the electrospun mat of polar polymers were amended into 3-D architecture using aqueous SB solution while methanol solution was found equally effective for both polar and non-polar polymers. Moreover, the fabrication process was fast in methanol

*Abbreviations:* Mw, Molecular weight; wt%, weight percent.

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solution compared to an aqueous solution due to the rapid liberation of hydrogen gas from the methanolysis reaction compared to the hydrolysis reaction. This process will reveal a new approach for the fabrication of a three-dimensional, low-density, nanofibrous materials for biomedical and industrial applications using a wide variety of polymers.



## 1. Introduction

Electrospinning is a remarkably simple, robust, and versatile technique capable of generating a nanofibrous membrane with high porosity and spatial interconnectivity of fibers to transport nutrients and wastes [1]. A scaffold based on electrospun nanofibers has a large specific surface area for incorporating bioactive molecules to facilitate efficient and selective cellular response [2]. Such scaffolds with various alignments have shown superior capacity for shaping cell morphology, guiding cell migration and affecting cell differentiation when compared to other types of scaffolds [3]. However, an electrospun mat has densely packed fiber layers, and the lack of space between adjacent layers creates a two-dimensional sheet-like structure with superficial planar pores [4–6]. Therefore, a major concern of electrospun scaffolds is that they have only a superficially porous network, resulting in a sheet-like framework that restricts cell infiltration and growth into the scaffolds [6]. Thus, new procedures are still required to fabricate a three-dimensional (3-D) nanofibrous and porous architectures that better mimic the configuration of native extracellular matrix and guide tissue regeneration.

Many efforts have been made to develop 3-D electrospun scaffolds with loosely packed nanofibers [6–11]. Park et al. [5] prepared macroporous nanofibrous scaffolds by concurrent electrospinning and deposition/leaching of salt particles. Mauck and coworker [6] prepared 3-D porous scaffold by co-electrospinning of the desired polymer with easily water-soluble material and dissolving it out, while Ekaputra et al. [7] electro-sprayed hydrogels into the scaffolds during electrospinning and selectively leached the water-soluble gel to create a cell-permeable fibrous mesh. In another study, Jun and coworkers modified the electrospinning system to fabricate a cotton ball like uncompressed electrospun scaffold [6]. Multilayered 3-D scaffolds have been studied for varieties of tissue engineering applications such as meniscus tissue engineering [12], cardiac tissue engineering [13]. Such multilayered porous 3-D scaffolds are more effective for cell activity than other 3-D structures. Therefore, recently, Stamatialis et al. [14] reported a 3-D scaffold consisting of stacked multilayered porous sheet for tissue engineering. They found that the inter-porosity of the sheets allows diffusion of nutrients and signaling product between the layers and existing microchannel facilitate nutrient supply on a layer as they provide space for the culture medium perfused throughout the scaffold. Similarly, Kon et al. [15] reported a multilayer gradient nano-composite scaffold for osteochondral regeneration where they found that such matrix are suitable to direct and coordinate the process of bone and hyaline-like cartilage regeneration. Moreover, multilayered structure are not only studied for tissue engineering applications but also for the analysis of viability and smooth muscle alpha actin expression [16]. Mikos et al. [17] reported that cell infiltration and growth in a multilayered scaffold depends upon the thickness of each layer; increasing the thickness of nanofiber layer reduced the cells infiltration of into the scaffold. These strategies required special manufacturing protocols, still do not offer a thin, multi-layered architecture of nanofibers that might be effective for cell proliferation and growth.

Nevertheless, gas foaming techniques exhibit a strong advantage, especially in the processing of cellular polymers for various biomedical and industrial applications [18,19]. The gas foaming process utilizes the nucleation and growth of gas bubbles that are generated *in situ* either via chemical reaction or by adding inert gases to the polymer phase at different physical environments [20], still this versatile technique is not reported for the processing of electrospun nanofibrous mat. Electrospun membranes have densely packed nanofibers with interconnected micro/nano pores. Generation of gas bubbles *in situ* into the pores of the nonwoven electrospun mat via chemical reaction could be a potential strategy to reorganize the nanofibers in a loosely packed, 3-D architecture. Therefore, we are motivated to develop a gas foaming technique for the fabrication of 3-D electrospun nanofibrous scaffold using *in situ* gas foaming process.

The present study introduces a novel and facile technique to construct a 3-D nanofibrous scaffold by post-electrospinning procedures via *in situ* gas foaming process. Sodium borohydride (SB) solution is used as a gas foaming reagent. Electrospun mats of different polymers were subjected to gas foaming process to understand the fabrication technique. Digital images, FE-SEM images, mercury porosimetry, XRD, and FT-IR spectroscopy were used to distinguish the morphology and structure of the pristine electrospun mat and SB-treated mat. We believe, the proposed novel post-electrospinning procedure may become a superior and simple method to fabricate a 3-D low-density, electrospun-based materials in desired shapes and size for biomedical and industrial applications using a wide variety of polymers.

## 2. Experimental

### 2.1. Preparation of electrospinning solutions

Poly (ε-caprolactone) (PCL, Mw = 70,000–90,000), Nylon-6 (N6, Mw = 11,202), Cellulose acetate (CA, Mw = 30,000), and polyvinylidene fluoride (PVDF, Mw = 1,800,000) were purchased from Sigma–Aldrich (USA). A N6 solution (22 wt%) was prepared by dissolving N6 pellets in a solvent system of formic acid and acetic acid (analytical grade, Showa, Japan) (4:1 w/w) under magnetic stirring for 12 h at room temperature. PCL solution (10 wt%) was prepared by dissolving in dimethylformamide (DMF, Sam Chun, Korea) and 2-butanone (MEK, extra pure, Sam Chun) (4:1 w/w), and CA solution (17 wt%) was prepared dissolving in a mixture of acetone (Samchun, Korea) and N,N-dimethylacetamide (DMAc, Samchun, Korea) (3:1 w/w). PVDF solution (15 wt%) was prepared by dissolving in solvent mixture of acetone/DMAc (7:3 w/w).

### 2.2. Electrospinning setups and procedure

A newly introduced robot-controlled electrospinning setup was used in this study (Fig. S1). The electrospinning solution (approximately 8 mL) was drawn into a 12-mL plastic syringe connected with a metal capillary (di = 0.51 mm, 21 G) through a plastic tube. It was loaded onto a syringe pump under digital control and the flow rate was maintained at 0.5 mL/h for nylon-6 and PVDF, and 1 mL/h for CA and PCL separately. Electrospinning was performed at room conditions (temperature = 26 °C, and humidity = 44%) where the parameters include applied voltage of 20 kV for N6