ABSTRACT

Fabrication and Characterization of Three-Dimensional Nano-Fibrous Scaffolds for Tissue Engineering Applications

Mahesh Kumar Joshi
Department of Bionanosystem Engineering
Graduate School
Chonbuk National University

In the past decade, considerable efforts have been made to fabricate the biomimetic scaffolds from electrospun nanofibers for tissue engineering applications.Electrospinning technique offers unique advantages in the production of tissue engineering scaffolds compared to other methods in terms of simplicity, high surface-to-volume ratio scaffolds and process versatility. As promising as it may seem, this technology is still in its infancy, and further development is critical before it can be used for any practical biomedical applications. One of the major concerns with electrospunnanofibrous scaffolds is that they have only a superficially porous network, resulting in a sheet-like two-dimensional (2D) framework that restricts cell infiltration and growth. Moving towards the next generation of electrospun scaffolds, increasing research efforts are being focused on issues such as three-dimensionality, bio-functionalization, and improved biomechanical properties of the scaffolds.

The research project outlined in this dissertation was aimed to address the first two issue mentioned. To do so, a novelpost electrospinning process isdeveloped for the modification of two dimensional (2D) electrospun membrane into macro-porous, multi-layered, low-density, three-dimensional (3D) scaffolds. In situ gas foaming process is explored for the post electrospinning modifications. The theoretical model was developedto fabricatethe 3D scaffolds and then validated with experimental findings. Sodium borohydride was used as gas foaming agent. Hypothesis is that when electrospun membranes immerged in sodium borohydride solution, the interconnected pores will be filled with the SB solution driven by capillary forces where SB solution will undergoes hydrolysis producing hydrogen gas. The gas molecules generated in-situ in the pores will form clusters to minimize the free energy resulting in pore nucleation that will reorganizes the nanofibers to form a low density, macro-porous, spongy, and multi-layered 3D scaffold. To validate the hypothesis, electrospun membranes of various polar and non-polar polymers were treated with SB solution varying the different parameters. 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 3D 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 solution compared to an aqueous solution due to the rapid liberation of hydrogen gas from the methanolysis reaction compared to the hydrolysis reaction.Experimental results showed that as- fabricated 3D scaffolds have excellent ability for cell infiltration, proliferation and growth. This method proved to be significantly better than other modifications methods attempted earlier.

Next part of this dissertation deals with the fabrication of 3D cellulose sponge for tissue engineering applications. Cellulose is an almost inexhaustible biopolymer that has been used in a number of industries due to its eco-friendly characteristics. Recent developments in cellulose research show that it is a promising biomaterial for tissue engineering, stem cell research, and regenerative medicine. Bacterial-produced cellulose is primarily studied for bone regeneration. However, it does not offer the ability to control the fibers on the nanoscale or microscale, which limits its applicability in tissue engineering. Due to strong inter- and intra-molecular interactions that originate from the hydrogen bonds and rigid backbone structure, cellulose does not melt or dissolve in conventional solvent systems which makes difficult for electrospinning. Compared to cellulose, cellulose acetate (CA), precursor of cellulose, is easy to process and has good spinnability. Taking the advantage of the expanded processability window of CA, cellulose fibers are produced through alkaline saponification of CA fibers. Thus produced cellulose fibers were treated with SB solution modifying into 3D cellulose sponge. As-fabricated 3D cellulose sponge showed better cell infiltration, growth and proliferations compared to the cellulose and cellulose acetate membranes.

Addressing the second issue of bio-functionalization, cellulose-synthetic hybrid fibers were fabricated. Electrospun membrane of synthetic polymers such as PCL and N6 are widely studied for tissue engineering applications. However, the poor wettability and hydraulic permeability of the membranes hinder their applications in tissue engineering applications. To enhance the biocompatibility and physicochemical properties of such synthetic polymers, cellulose-synthetic polymer composite fibers were fabricated. Cellulose acetate (CA) was blended with different synthetic polymers (PCL and N6) in various mass ratios, and nonwoven hybrid fibers were fabricated using an

electrospinning process. CA content of the hybrid fiber was transformed into cellulose (CL) by post-electrospinning treatment via alkaline saponification. The effect of the mass composition and subsequent saponification on the nanofiber morphology as well as physicochemical properties such as mechanical strength, crystallinity, surface wettability, bio-mineralization, and biocompatibility were determined. Regeneration of cellulose chains in the nanofibers increased the number of hydroxyl groups, which increased the hydrogen bonding, thereby improving the mechanical properties and wettability of the composite nanofibers. The improved wettability and presence of surface functional groups enhanced the ability to nucleate bioactive calcium phosphate crystals throughout the matrix when exposed to a simulated body fluid solution. Cellulose- synthetic hybrid fibers were found to be more thermally stable than pristine polymer (PCL and N6) nanofibers. Cell viability assay and microscopy imaging revealed that the cellulose-synthetic hybrid fibers have excellent cell proliferation and spreading compared to the pristine fibers.

In conclusion, the work presented in this dissertation provided a method of fabricating the next generation of electrospun scaffold capable of 3D tissue integration and improved physicochemical properties. Such a technological advancement will prove advantageous in achieving improved tissue regeneration and repair.

Keywords: Electrospinning; 3D Scaffolds; tissue engineering; post electrospinning process; gas foaming; alkaline saponification

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ratios of PCL to CA, respectively, and MR1, MR2, MR3, and MR4 represent the saponified mats corresponding to M1, M2, M3, and M4, respectively. Scale bar in FE-SEM images is 4μm).

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Figure 6.17. SEM images showing cell attachment on different scaffolds; (a, b) PCL/CA, (c, d) PCL/CL, and (e, f) SBF-incubated scaffolds at three days (a, c, e) and seven days (b, d, f)

Figure 6.18. Confocal microscopy. Cells on scaffolds were stained with DAPI to display blue (nuclei) and ActinGreen™ 488 to display green (cytoskeleton). MC3T3-E1 activity on different scaffolds (a, b) PCL/CA, (c, d) PCL/CL, and (e, f) SBF-incubated PCL/CL mat. (a, c, e) represent three days and (b, d, f) represent seven days incubation.

Figure 7.1. FE-SEM images of the different mats. (A) Before saponification: (i) pristine N6, (ii) N6-90/CA-10, (iii) N6-75/CA-25, (iv) N6-50/CA-50. (B)

List of Abbreviations

2D -	Two dimensional
3D -	Three Dimensional
BSA -	Bovine Serum Albumin
ALP -	Alkaline Phosphatase
ARS -	Alizarin Red S
CaL-	Calcium Lactate
CaP-	Calcium phosphate
CL-	Cellulose
DAPI -	4',6-diamidino-2-phenylindole
DCM-	Dichloromethane
DMEM -	Dulbecco's Modified Eagle Media
DMF-	Dimethylformamide
DMAc-	Dimethylacetamide
DSC -	Differential Scanning Calorimetry
EBM-2 -	Endothelial Basal Media-2
ECM -	Extracellular Matrix
EDX -	Energy Dispersive X-Ray Spectroscopy
FBS -	Fetal Bovine Serum
HAp -	Hydroxyapatite
LA-	Lactic acid
IR-	Infra red
LCSM -	Laser Scanning Confocal Microscope
Mw-	Molecular weight
N6-	Nylon 6
PBS -	Phosphate-buffered Saline
PCL -	Poly (ε-caprolactone)

PEO -	Polyethylene oxide
PLA -	Polylactic acid
PVA -	Polyvinyl alcohol
SB-	Sodium borohydride
SBF-	Simulated body fluid
SEM -	Scanning Electron Microscopy
TEM-	Transmission electron microscopy
TGA-	Thermogravimetric analysis
Tc-	Crystallization peak temperature
Toc-	Crystallization onset temperature
Tom -	Melt onset temperature
T_m -	Melt peak temperature
Tg -	Glass Transition Temperature
Tm -	Melting Temperature
UV -	Ultraviolet
XRD-	X-ray diffraction
XPS-	X-ray photoelectron spectroscopy

List of Symbol

μ - micro

ε - epsilon

β - beta

α - alpha

ΔH_m - melting enthalpy

ΔHc - crystallization enthalpy