Fabrication and Characterization of Nanofibrous Scaffolds for Corneal Tissue Engineering Applications

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by

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Chapter 4

Conclusions and future scope of work

The primary aim of this thesis was to select an affordable, environment-friendly biomaterial and to fabricate nanofibrous scaffolds by utilizing enabling technologies for corneal tissue engineering applications. Therefore, to accomplish the goals mentioned, the present thesis demonstrates the development and fabrication of various three-dimensional structures using an electrospinning approach. Because the vast majority of the corneal stroma is comprised of different kinds of collagen arranged in a complex architecture, collagen biomaterials are the most appropriate materials for the fabrication of corneal equivalents. However, because of the high cost of collagen, its inexpensive hydrolysate form i.e. gelatin may be used to create scaffolds comparable to an extracellular matrix in place of the collagen. Since gelatin could be hydrolyzed in two different ways, it is critical to choose an appropriate gelatin material for the fabrication of nanofibrous scaffolds. Therefore, a comparative study was performed to determine the suitable gelatin type for the fabrication of nanofibrous scaffolds as corneal stromal analogs.

The dissertation applies novel methods and emphasizes electrospinning techniques using natural polymers to fabricate nanofibrous scaffolds that may serve as corneal stromal equivalents. In an attempt to select the gelatin type for scaffold fabrication, we compared electrospun nanofibers of both gelatin types A and B. The study reports gelatin A nanofibrous scaffold as a superior candidate compared to gelatin type B nanofibrous scaffold for further exploration. Unlike conventional cell culture substrates, these platforms facilitate cell adhesion and spreading in all the three dimensions as well as provide high porosity for the culture medium and other nutrients to diffuse. Moreover, these platforms possess less stiffness values as compared to the two-dimensional (2D) culture platforms. Addition of silk nanofibers followed by physical cross-linking of the scaffolds with ethanol vapor furnishes

them with greater stability and integrity to maintain the tissue formation for a longer duration as well as the capability to biodegrade at a rate comparable to the formation of native tissues. Biocompatibility of such microengineered platform is tested in vitro which exhibits a better cell performance in terms of its growth and proliferation.

The thesis also focuses on the development of three-dimensional biodegradable scaffolds from natural protein biomaterials such as gelatin and silk. It demonstrates the ‘green crosslinking’ technology to crosslink the silk physically and enhance its strength. Term ‘green crosslinking’ employs to those kinds of crosslinkers or crosslinking techniques that are natural/non-toxic and environment-friendly. For example, Dastidar et al. demonstrated a fully ‘green’ water based process to crosslink corn (cereal) and potato (tuber) starch in order to enhance mechanical properties as well as lower hydrophilicity (Ghosh Dastidar and Netravali 2012). Similarly, Chen et al. utilized non-toxic polybasic organic acids (maleic acid, tartaric acid, citric acid, and malic acid) to green-crosslink the chitosan (CS)/poly(vinyl alcohol) (PVA) composite nanofibers to prepare a suitable scaffold for tympanic membrane repair (Chen et al. 2022). The thesis also reveals the ability of composite reinforcement technique to fabricate nanofibrous scaffolds that enhance the mechanical transparency and stability of the fabricated scaffold without losing the optical transparency. The method of physical crosslinking of silk fibroin protein enhances the strength and integrity of the scaffold.

Following is a brief comparison between two constructs developed using the electrospinning approach. Both the nanofibrous scaffolds exhibited acceptable output after physically crosslinking using ethanol vapour treatment compared to their uncrosslinked counterparts.

However, there were a few variations in the properties of the fabricated nanofibrous scaffolds based on numerous parameters.

Scanning electron microscope (SEM) micrograph of both the scaffolds demonstrated mesh of randomly oriented nanofibrous structure. However, in case of nanofibrous scaffolds permeated with silk fibroin (SFG) the permeation of silk left some beady appearance on the surface periphery whereas the gelatin-permeated SF (in formic acid; T) scaffold exhibited a bead-free nanofibrous composite scaffold. It was found that the water retention capacity of both scaffolds was almost the same, ranging from 800-1000%. The lysozyme degradation test resulted in 40% degradation of SFG scaffold whereas, gelatin-permeated SF (in formic acid; T) scaffolds showed high stability under physiological conditions i.e., ~25% compared to SFG scaffold. Transparency percentage of gelatin-permeated SF (in formic acid; T) scaffold (~77%) was significantly higher than the transparency of SFG scaffold (~60%); showing comparable transparency with respect to adult rat cornea (~78%). Cell culture and MTT assays of both the scaffolds exhibited good cellular compatibility and proliferation wherein SFG and gelatin-permeated SF (in formic acid; T) scaffolds demonstrated ~72% and 77% proliferation by 6^{th} day of culture. Overall, the gelatin-permeated SF (in formic acid; T) scaffold outperformed the SFG nanofibrous scaffold in terms of stability under physiological conditions, transparency, cellular compatibility and proliferation.

In this thesis, we have attempted to mimic the stroma layer of the cornea, which constitutes the major part of cornea. The corneal stroma accounts for 90% of the total thickness of the corneal epithelium. The corneal stroma is mostly composed of water (78% water or 3.5g H_2O/g dry weight). Glycosaminoglycans (GAGs) are the most abundant heteropolysaccharides in the corneal stroma (Pacella et al. 2015). As a result of its high

glycosaminoglycan content, the corneal stromal tissue layer has a significant propensity to swell when cultured under optimum culture (Polisetti et al. 2021). Furthermore, several studies have demonstrated that the epithelium and endothelium of the cornea are the limiting layers for the extent of swelling of cornea, because of the presence of active ion transport sites that govern the hydration level of the hydrophilic stroma (Edelhauser 2006). Therefore, it is unfair to interpret extent of swelling in absence of epithelial and endothelial layer. Moreover, in the present thesis, the swelling percentage of the scaffolds was examined in PBS solution, I believe that if the same scaffold would exist in ocular environment where the surrounding liquid is viscous in nature may cause decrease in the swelling percentage. When the cornea is injured by an external agent, such as a chemical or traumatic injury, the ion transport system inside the external layers of cornea is disrupted, resulting in corneal swelling, which affects the mechanical, optical, and functional properties of the cornea i.e., the cornea loses its transparency as a result of the high diffraction of light entering the swollen cornea (Dawson and Edelhauser 2010). Furthermore, swelling in the cornea may result in the infiltration of leukocytes into the stroma, which might result in inflammation inside the cornea (Labelle 2017). Moreover, it is important to note that it is the first attempt in the literature for quantification of swelling behavior of nanofibrous scaffolds fabricated in view of corneal tissue engineering applications. Moreover, the fabricated scaffold possesses tunable swelling property, we would endeavour to tune the swelling property of the fabricated nanofibrous scaffold in future.

The future perspectives of the current research work may involve testing of additional chemicals or other natural crosslinkers for the fabrication of composite nanofibrous scaffolds and determine the comparative efficacy of their crosslinking in terms of corneal equivalent

construction. The observations gathered from the investigation carried out to compare different scaffold fabrication techniques utilizing gelatin and silk may be used for comparing the results obtained from other processes of scaffold fabrication techniques. Moreover, the use of nanotechnology in silk and gelatin-based electrospun nanofibers can potentially be a breakthrough technological advancement in the field of scaffold fabrication. Various other natural biomaterials in combination with gelatin/silk could be employed to fabricate novel corneal stromal equivalents and can be compared with the present composite scaffolds. The fabricated scaffolds could be tested in vivo to check the biocompatibility and suitability of the fabricated scaffold in terms of mechanical stability, transparency, stability, and degradation with respect to cornea. Electrospun gelatin and silk nanofibers have not been much explored in the field of corneal tissue engineering for recreation of corneal equivalents, which leaves scope for further exploration.

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