PREFACE

The cornea is the eye's outermost layer and together with the eyelids and sclera protects the interior of the eye. The cornea accounts for 75% of the eye's refractive power and it is transmitting almost all visible light into the eye. It is a thin, elastic, non-vascularized tissue that adjusts the curvature of the eye and comprised of five separate layers and three distinct cell types. Bilateral corneal blindness affects over 10 million people globally. Corneal disease, second only to cataracts, is a leading cause of blindness. Currently, the only way to restore eyesight is via corneal transplantation. Total corneal transplantation has a 90% success probability in individuals with favorable prognoses (minimal graft vascularization and inflammation), but virtually no chance in patients with alkali burns or repeated graft failures. Among the disadvantages of corneal transplantation, include high rates of immunological rejection, the risk of infection and donor scarcity.

As a viable alternative to donated corneas, a tissue-engineered cornea stromal replacement may provide substantial advantages. Tissue engineering has emerged as a potentially wide field that may result in the creation of synthetic corneal stromal equivalent tissues. The approach faces several difficulties, including the following: (1) selecting the type of corneal cell and its isolation; (2) developing a suitable biomaterial scaffold that promotes cell growth and differentiation; and (3) replicating the intricate, sophisticated architecture of the tissue with uniformly aligned architecture, resulting in corneal tissue with optimal transparency, refractive power, and mechanical stability. (4) Recreating a non-vascularized and innervated environment that guarantees the tissue's survival by sustaining the tissue's high metabolic requirement while also maintaining transparency.

The enabling scaffold fabrication technologies for tissue engineering open new possibilities to mimic the native microenvironment of a cell suitable enough to contribute to the overall functions of a particular cell or tissue. In the present thesis, we have developed various functional three-dimensional micro- and nanoscale structures using electrospinning approach. Collagen biomaterials are the most suitable materials for fabricating corneal analogues because the vast majority of the corneal stroma is composed of various types of collagen organized in an intricate architecture. Due to the high expense of collagen, its hydrolysate gelatin may be utilized in lieu of the collagen to form an extracellular matrix. Due to the fact that gelatin may be hydrolyzed in two distinct ways, it is essential to utilize an appropriate form of gelatin while fabricating nanofibrous scaffolds. As a result, a comparative research is performed to identify which form of gelatin should be utilized in the construction of nanofibrous scaffolds. To the best of our current knowledge, there remains no reports concerning the study related to acid hydrolyzed gelatin (gelatin A) and alkaline hydrolyzed gelatin (gelatin B) nanofibrous scaffolds together. We have made an attempt to compare these gelatin based nanofibrous scaffolds that are constructed with the use of a ternary solvent (glacial acetic acid/ethyl acetate/water). Electrospinning methods provide gelatin scaffolds a high surface-to-volume ratio as well as a high porosity. The electrospun scaffold based on gelatin A has a water retention capacity of about 800% and is found very stable at ambient temperature. Similarly, its transparency is similar to that of adult rat cornea, making it a viable option for corneal tissue growth.

In addition, without crosslinking the gelatin A nanofibrous sheet is found incapable of enduring physiological circumstances on its own for longer duration. As a result, penetration of silk into the gelatin scaffold followed by physical crosslinking (with ethanol vapor) significantly increases its stability. The nanofibrous gelatin A scaffold

permeated with silk fibroin (SFG) provides optimal stability as well as proliferation for corneal fibroblast cells close to 72% compared to that of the 5th day control. Chemical or enzymatic cross linkers may be used to further adjust the degradation rate and stability depending on the application. Thus, the comparison results indicate that the SFG scaffold is more stable than gelatin A alone; enabling it potentially helpful in corneal tissue engineering application.

We also present the successful development of a new nanocomposite gelatin hydrogel system reinforced with mechanically robust silk nanofibers. The permeation of gelatin into the silk nanofibrous scaffold improved the proportion of visible light transmission, increasing its usefulness in a variety of applications where transparency is critical, such as skin wound dressing and most significantly ocular tissue constructions. Ethanol vapor treatment of silk nanofibers resulted in the change of the less stable random helical conformation to the more stable sheets shape. Scanning electron microscopy (SEM) micrographs were used to examine the micro/nano-scale characteristics of the constructed scaffolds. Fourier transform infrared spectroscopy (FTIR) showed characteristic peaks associated with polymeric functional groups and their changes after ethanol vapor treatment. The liquid retention capacity of gelatin-permeated SF (in formic acid; T) [T= Treated] was found to be significantly greater than that of SF (in aqueous; T) and SF (in formic acid; T). All scaffolds were shown to be substantially stable for up to 14 days when incubated in phosphate buffered saline (PBS) at 37°C, except the untreated SF (in aqueous; NT) [NT= Non-treated]. In comparison to untreated samples, treated samples demonstrated substantially improved physical and microscopic stability. The degradation analysis showed that all ethanol-treated samples exhibited improved and enhanced stability. The cytocompatibility of corneal fibroblast SIRC cells and L929-RFP fibroblast cells was shown to be much higher in the gelatin-permeated SF (in formic acid; T)

scaffold than in the other scaffolds. The novelty of this study is the creation of a nanofibrous silk-gelatin composite scaffold that exhibits superior transparency, mechanical strength and cellular biocompatibility. Overall, the results indicated that the addition of gelatin to nanofibrous silk scaffolds resulted in durable, highly cytocompatible and transparent scaffolds; making them an excellent option for corneal tissue engineering. Although the material was effectively characterised in vitro in the current research, further extensive in vivo characterization is needed to verify the suggested materials for creating corneal stromal equivalents. Electrospun gelatin and silk nanofibers have received little attention in the area of corneal tissue engineering for the purpose of recreating corneal equivalents, leaving space for future research.

To summarize, the present thesis demonstrates the development and fabrication of various three-dimensional microscale structures employing the enabling electrospinning technology.

Keywords:

Cornea, gelatin, silk fibroin, electrospinning, tissue engineering, scaffolds, extracellular matrix.