Burns, persistent wounds, and severe accidents may cause extensive skin loss, resulting in lifelong impairment of skin functions. The gold standard treatment approach for full-thickness wounds at present is a skin graft or split-thickness autologous skin transplant. However, many patients lack sufficient intact skin for such a treatment, and scar development renders this a cosmetically ineffective choice. Because of this, there is a demand for a tissue-engineered construct that promotes healing, prevents infection, and has a long shelf life.

The enabling scaffold fabrication methods for tissue engineering provide new opportunities to imitate the natural milieu of a cell sufficiently well to assist in the overall functions of a specific cell or tissue. In this thesis, we used electrospinning, freeze-thawing/cryogelation, and salt-leaching to make a variety of functional structures on the micro and nano scales.

The first part of the thesis presents the development of biocompatible soy protein isolate/silk fibroin (SPI/SF) nanofibrous scaffolds through electrospinning a novel protein blend SPI/SF. Prepared nanofibers were treated with ethanol vapor to obtain an improved water-stable structure. Fabricated scaffolds were characterized through scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), UV–VIS spectrophotometry and image analysis. The scaffolds were found significantly stable for a prolong duration at the room temperature as well as at 37 °C, when placed in phosphate buffered saline (PBS), nutrient medium, and lysozyme-containing solution. The potential of fabricated scaffolds for skin tissue regeneration was evaluated by in vitro culturing of standard cell lines i.e., fibroblast cells (L929-RFP (red fluorescent protein) and NIH-3T3) and melanocytes (B16F10). The outcomes revealed that all the fabricated nanofibrous scaffolds were non-toxic towards normal mammalian cells. In addition, healing of full-thickness wound in rats within 14 days after treatment with a nanofibrous scaffold demonstrated its suitability as a potential wound dressing material. Interestingly, we found that nanofibers induced a noticeable reduction in the proliferation rate of B16F10 melanoma cells.

In the second part of the thesis, we demonstrate the development of polyvinyl alcohol (PVA) and SPI based scaffolds by physical crosslinking using the freeze-thaw method. PVA/SPI ratio was varied to examine the individual effects of the two constituents. The physicochemical properties of the fabricated scaffolds were analyzed through FTIR, SEM, X-ray diffraction (XRD), TGA and differential scanning calorimetry (DSC). SPI concentration significantly affected the properties of scaffolds, such as the extent of gelation (%), pore size, porosity, degradation, swelling and surface wettability. The in vitro degradation of fabricated hydrogels was evaluated in PBS and lysozyme solution for a duration of fourteen days. The in vitro compatibility of prepared hydrogels was evaluated by MTT assay with NIH-3T3 cells (fibroblast). The water vapor transmission rate (WVTR) assays showed that all hydrogels possessed WVTR values in the range of 2000 to 2500 g $m^{-2}day^{-1}$, which is generally recommended for ideal wound dressing. Overall, the obtained results reveal that the fabricated scaffolds have excellent biocompatibility, mechanical strength, porosity, stability and degradation rate, and thus carry enormous potential for tissue engineering applications. Furthermore, full-thickness wound healing study performed in rats supported them as a promising wound dressing material.

The third part of the thesis presents the fabrication of chemically cross-linked highly porous SPI cryogels. Herein, glutaraldehyde (GA) cross-linked macroporous sponge-like SPI scaffolds were prepared using the cryogelation technique for tissue engineering applications. The prepared SPI cryogel scaffolds possess an interconnected porous structure with a high porosity with pore sizes in the range of micrometres. The physicochemical properties of the fabricated scaffolds were analyzed through FTIR, SEM, TGA and DSC. The morphology, porosity, swelling capacity, and degradation rate of the cryogels were found to be dependent on the concentration of polymer to crosslinking agent. All cryogels showed elasticity and physical integrity in cyclic compression analysis, as these matrices restored their original length after being compressed to one-fifth of their original length. These cryogels showed excellent mechanical properties, immediate water-triggered shape restoration and absorption speed. Furthermore, cryogels outperformed cotton and gauze in terms of blood clotting and blood cell adherence. The in vitro and in vivo studies demonstrated the potency of SPI scaffolds for skin tissue engineering applications. Our findings showed that crosslinking with GA had no detrimental effects on cell viability. In addition, an in vivo full-thickness wound healing study in rats validated them as good potential wound dressing materials. Overall, we prepared superporous, non-toxic, stable soy protein cryogels that could be useful in biomedical applications such as cell culture matrices and the replacement of damaged tissue, specifically as a wound dressing.

Besides, in the last part of the thesis we have proposed a platform for topical wound dressing material using a polydimethylsiloxane (PDMS) scaffold in order to enhance the skin healing process. In vitro co-culture assessment of epidermal-origin mouse B16-F10 melanocyte cells and mouse L929 fibroblast cells in three-dimensional polymeric scaffolds has been carried out towards developing bio-stable, interconnected, highly macroporous, PDMS based tissue-engineered scaffolds, using the salt leaching method. To determine a suitable ratio of salt to PDMS pre-polymer in the scaffold, two different samples with ratios 2:1 and 3:1 [w/w], were fabricated. Effective pore sizes of both scaffolds were observed to lie in the desirable range of 152–165 μm. In addition, scaffolds were pre-coated with collagen and investigated as a podium for culturing the chosen cells (fibroblast and melanocyte cells). Experimental results demonstrate not only a high proliferative potential of the skin tissue-specific cells within the fabricated PDMS based scaffolds but also confirm the presence of several other essential attributes such as high interconnectivity, optimum porosity, excellent mechanical strength, gaseous permeability, promising cell compatibility, water absorption capability and desired surface wettability. Therefore, scaffolds facilitate a high degree of cellular adhesion while providing a microenvironment necessary for optimal cellular infiltration and viability. Thus, the outcomes suggest that PDMS based macroporous scaffold can be used as a potential candidate for temporary skin dressing material. In addition, the fabricated PDMS scaffolds may also be exploited for a plethora of other applications in tissue engineering and drug delivery. It can be used as a temporary upper layer in a bilayer skin substitute.

To summarize, the current thesis demonstrates the development and fabrication of a variety of three-dimensional microscale structures by employing the enabling technologies of tissue engineering such as electrospinning, freeze-thawing/cryogelation, and salt-leaching. Overall, the findings of these studies indicate that the developed materials have the potential to be used in the fields of soft tissue engineering and wound healing.

Keywords:

Skin, soy protein isolate, silk fibroin, polyvinyl alcohol, polydimethylsiloxane, electrospinning, freeze-thawing, cryogelation, salt-leaching, tissue engineering, scaffolds, wound dressing.