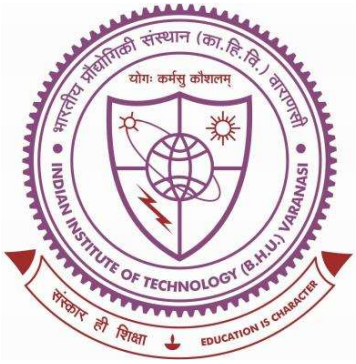
Fabrication and Characterization of Three-dimensional Functional Scaffolds for Skin Tissue Engineering



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Award of Degree

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by

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Chapter 6

Conclusions and Future Scope of Work

This thesis primarily focused on the fabrication and characterization of novel biomaterial scaffolds in a variety of morphologies for wound healing and TE applications. To achieve the aforementioned objective, the current thesis examined the development and construction of diverse 3D structures using advanced TE techniques such as electrospinning, freeze-thawing, cryogelation and salt-leaching.

In the second chapter of the thesis, electrospun nanofibrous scaffolds of SF and SPI blend using formic acid as a solvent were successfully developed. SEM micrographs of all nanofibrous scaffolds showed nanofiber diameters between 30 and 360 nm and ~50 % porosity. Nanofibrous scaffolds were found stable in an aqueous medium (PBS/lysozyme containing solution/DMEM medium) for up to 20 days. The degradation study showed that we could change how fast the nanofibers degrade by changing the ratio of polymers. This study also demonstrated that EtOH vapor treatment induce the conversion of the random coil structure to the β sheet structure in SF, which provided enhanced stability to the scaffold but at the cost of transparency. The fluorescent and SEM microscopic analysis of cultured fibroblast cells, as well as the MTT assay, were used to investigate the cellular biocompatibility of nanofibrous scaffolds toward mammalian cells. Excellent growth of ≥ 80% was observed after four days of culture. Furthermore, in vivo wound healing in rats demonstrated the applicability of the SPI/SF nanofibers as a potential wound dressing material. SPI in combination with SF produced by electrospinning is an economical and feasible alternative to producing nanofibers by electrospinning. Therefore, we anticipate that the prepared SPI/SF blend electrospun nanofibers could be an attractive material for many TE applications, especially for accelerating wound healing.

The thesis also focused on the development of physically cross-linked PVA and SPI composite hydrogels using the freeze-thawed method. The addition of SPI to PVA

hydrogels significantly increased their porous architecture, degradation, pore size, tissue adherence, cell infiltration, and growth. However, an increase in SPI concentration led to a significant reduction in the mechanical strength and gelation percentage. In particular, a 50% SPI addition led to up to a 10-fold increase in the swelling ability of the PVA hydrogels, while the gel fraction decreased to 85.8 ± 1.7 from 96 ± 0.9% (100% PVA). The average pore size increased from 0.64 ± 0.43 μm for 100% PVA samples to 15.35 ± 13.9 μm for 50/50 PVA/SPI samples. The value of the thermal degradation temperature of 100% PVA increased from 305.5 to 332˚C upon addition of SPI. Thus, the thermal stability of PVA hydrogels increased after the addition of SPI. An excellent growth of ≥ 60% after 4 days of culture was observed for 70/30 PVA/SPI and 50/50 PVA/SPI samples. In-vivo wound healing in rats showed that PVA/SPI scaffolds could be used as a possible wound dressing material. Because of this, we believe that the PVA/SPI composite scaffold that we have prepared could be an appealing material for many TE applications, particularly for enhancing the wound healing rate.

In the next part of the thesis, chemically cross-linked superporous SPI sponges using the cryogelation technique were successfully prepared. The method of chemical crosslinking of SPI using the cryogelation technique enhanced the strength and integrity of the scaffolds. SPI cryogels outperform traditionally prepared hydrogels in terms of flexibility, rapid, reversible transformation in 3D microstructure response to external factors, and outstanding swellability in aqueous media. They encourage proper cellular migration during growth and can act as a regenerative template in TE. As a result, we anticipate that the prepared SPI scaffold will be appealing for many TE applications, particularly for accelerating wound healing. The other possible applications of prepared SPI cryogels are wound dressing, drug delivery, tooth extractions, oral and maxillofacial surgeries, soft-tissue biopsies, dental implant surgeries, and agriculture and horticulture.

In the next part of the thesis, we developed macroporous PDMS scaffolds that can serve as a promising material for acellular and cellular tissue constructs. In particular, highly flexible, porous PDMS scaffolds with varying porosity were successfully fabricated using the salt-leaching method. The obtained porous PDMS scaffolds were found to be compatible with mammalian cells (B16-F10 melanocytes and L929 fibroblast cells), which shows their potential for TE applications. A significant level of porosity and interconnectivity between the pores facilitate media diffusion, cell attachment, and proliferation within the 3D environment. The fabricated scaffold are suitable for soft tissues, i.e., skin, cartilage, and blood vessels. Because of the inherent hydrophobic nature of the PDMS scaffold, it can be used as a wound dressing material that primarily behaves as a non-adherent film between wounded tissues and dressing. This makes it more suitable for atraumatic removal in order to prevent suffering and further tissue damage at the time of undressing. However, due to its poor degradation properties, it cannot be used in tissue regeneration applications where the construct must degrade over time. Therefore, PDMS scaffold can be used as a temporary wound dressing, upper layer of skin substitute and like a bandage.

The development of SPI-based biomaterials in a range of morphologies, which is the main focus of this thesis, is likely to open up a plethora of opportunities for future studies on this plant-based protein for various TE (soft and hard) applications.

In the future, the findings of this study as well as the knowledge gained about the development of SPI-based biomaterials, can be used as a framework for widespread use of plant proteins as green materials to replace animal proteins in a variety of biomedical applications. In this thesis, SPI/SF electrospun nanofibrous scaffolds have been found to be compatible with healthy cells while showing inhibitory effects or cytotoxicity toward melanoma cells (B16-F10 cells). Further investigations into the effects of SPI on different

cancer cells using in vitro cytocompatibility assays need to be considered. In vitro cell culture and in vivo wound healing studies performed in this thesis show that soy protein-based scaffolds could be a possible alternative to gelatin and collagen-based scaffolds. Our findings will aid in the quest for design of ideal wound dressings for tissue regeneration in the future.

The thesis also offers the opportunity to fabricate a novel, drug-preloaded, non-invasive, painless, and easy-to-apply PDMS-based flexible wound closure device. In vivo experiments can provide additional information about future applications of the fabricated PDMS scaffold. The findings of such studies will provide more information while validating their compatibility properties in comparison to their in vitro counterparts. Overall, this study lays the groundwork for future research centred on the use of the PDMS platform to develop tissue-engineered skin wound dressing. Our study also opens up the path to develop a bilayer skin substitute consisting of a PDMS layer and soy protein hydrogel or electrospun nanofibrous mat. Soy protein has not been much explored in the field of TE, which leaves room for further exploration.