ABSTRACT

Forwarded by

Prof. Pralay Maiti
(Co-Supervisor)
School of Materials Science and Technology
Indian Institute of Technology
(Banaras Hindu University)
Varanasi-221005

Prof. Nira Misra
(Supervisor)
School of Biomedical Engineering
Indian Institute of Technology
(Banaras Hindu University)
Varanasi-221005

Abstract

This thesis entitled "**Development of gelatin grafted poly(D,L-Lactide) based scaffolds for Biomedical Applications**" focuses on design and development of cell adhesive poly(D,L-Lactide) (PDLLA) based three dimensional (3D) scaffolds, evaluation of mechanical property of the 3D scaffolds and their application in tissue engineering.

To summarize, 3D porous matrix known as a scaffold has been used for variety of tissue engineering applications by introducing cells, or other biological molecules including genes, proteins, into it. Designing biomimetic scaffolds, which mimic the architectures of extracellular matrix (ECM) have become apparently a promising class of materials for tissue repair. The coherence in design of biomimetic scaffolds is based on mimicking natural ECM, which provides cells with a variety of physical, chemical, and biological cues that determine cell growth and function. Therefore, to create an optimized 3D structured tissue, biomimetic scaffolds are required to have controllable physicochemical, cell adhesion, and mechanical properties. Synthetic biodegradable polymer poly(lactic acid) (PLA) is widely used to fabricate 3D scaffolds for tissue regeneration due to its biodegradable nature, good mechanical properties, and importantly their approval its food and drug administration (FDA) for clinical use. However, the absence of cell adhering functional moieties in PLA limit its successful application in tissue engineering. Concerning to tailor the property of PLA, the work was designed to develop modified PLA and validate its cell proliferation efficiency and biocompatibility by *in vitro* and *in vivo* studies. The important results obtained are discussed below.

First, we tailored the cell adhesive properties of star shaped poly(D,L-Lactide) (ss-PDLLA) by grafting gelatin to its 4 arms. Grafting of gelatin on PDLLA backbone was confirmed by ^{1}H NMR and FTIR. The synthesized star shaped poly(D,L-Lactide)-*b*-Gelatin (ss-pLG) exhibited enhanced wettability and protein adsorption. The modification also facilitated better cell adhesion and proliferation on their respective polymer coated 2D substrates, compared to their respective unmodified ss-PDLLA. Further, 3D scaffolds were fabricated from gelatin grafted and unmodified polymers. The fabricated scaffolds were shown to be cytocompatible to 3T3-L1 cells and heamocompatible to Red Blood Cells (RBCs). Cell proliferation was increased upto 2.5 folds in ss-pLG scaffolds compared to ss-PDLLA scaffolds. Furthermore, a significant increase in cell number reveals, high degree of infiltration of cells into the scaffolds, forming a viable and healthy 3D interconnected cell community.

Surface and mechanical properties of the biomaterials are determinants of cellular responses. We also found that the synthesized ss-pLG possessing improved cellular adhesion and proliferation compared to unmodified ss-PDLLA. To better understand the relationship between the mechanical properties and cellular response, we established the cellular compatibility of gelatin-grafted PDLLA with respect to mechanical properties of biological tissues by comparing the ss-pLG and linear PDLLA-b-gelatin (l-pLG). In this view, l-pLG was synthesized and tissue-level compatibility of 1-pLG and ss-pLG was examined against fibroblasts (L929), myoblasts (C2C12) and preosteoblasts (MG-63). The cell proliferation of C2C12 was significantly higher within l-pLG scaffolds, whereas L929 showed intensified growth within ss-pLG scaffolds. The difference in cell proliferation may be attributed to the varying mechanical properties of scaffolds; where

the stiffness of l-pLG scaffolds was notably higher than ss-pLG scaffolds, most likely due to the variable levels of gelatin grafting on the backbone of PDLLA. In addition to that, a burst release of docetaxel (DTX) was observed from ss-pLG scaffolds compared to l-pLG scaffolds. Therefore, gelatin grafting can be used to modulate the mechanical property of the scaffolds and this study reveals the significance of the matrix stiffness to produce the successful 3D scaffolds for tissue engineering applications.

Additionally, we studied the ss-pLG against protein aggregation by examining the inhibition of amyloid fibrillation by protein grafted poly(D,L-lactide). Amyloids are group of proteins that are capable of forming amyloid fibrils, which is responsible for many neurodegenerative diseases including Alzheimer's disease (AD). In the present work, we studied ss-pLG against protein aggregation. Bovine serum albumin (BSA) was chosen as the model protein, which readily forms fibril under high temperature. We found that our synthesized ss-pLG efficiently suppressed the fibril formation of BSA compared to gelatin (Gel), which was evidently supported by Thioflavin T assay, circular dichroism (CD) spectroscopy and atomic force microscopy (AFM). In addition, ss-pLG significantly curtailed amyloid-induced hemolysis. We also found that incubation of ss-pLG with neuroblastoma cells (MC65) remarkably protected the cells from fibril-induced toxicity. The rescuing efficiency of ss-pLG was better than Gel, which could be attributed to the reduced lamella thickness in branched ss-pLG. Furthermore, we show the efficiency of ss-pLG to be used as a capping agent on gold nanoparticles ($AuNPs^{ss\text{-}pLG}$). Our study reveals the importance of grafting the natural protein *i.e.* gelatin to stabilize the physiological proteins and hence, ss-pLG could be used as a potential anti-amyloidogenic agent for the development of therapeutic agents.

Gelatin grafted PDLLA increased the hydrophilicity of the material as well as degraded at a faster rate. Inspired by nature, in this study hybrid materials were designed to increase the stability of the scaffolds, where organic and inorganic networks interpenetrate at the molecular level to achieve highly tailorable properties. We used 3-glycidoxypropyl trimethoxysilane (GPTMS) as a covalent cross-linker. This covalent interaction in h-Gel and h-ss-pLG increased the water contact angle. Further, the hepatocytes (Hep-G2) cell proliferation within h-ss-pLG scaffolds showed increasing trend till third day and after third day the rate of cell proliferation drastically decreased. This behavior may be due to the release of inorganic silica from the hybrid scaffolds that were evidenced by FTIR, energy dispersive X-Ray analysis (EDX) and elemental mapping. The subcutaneous implantation of Gel, ss-PDLLA and ss-pLG scaffolds into female rats showed very good compatibility and degraded completely after week 4, whereas the hybrid h-ss-pLG and h-Gel scaffolds showed relatively lesser infiltration of cells after week 4 which may be due to the release of silica and h-ss-pLG scaffolds were not degraded as they were covalently cross-linked with silane.

In conclusion, we anticipate the tailored gelatin grafted l-pLG and ss-pLG with improved cell proliferation property (when compared to unmodified l-PDLLA and ss-PDLLA) and enhanced anti-amyloidogenic property will be suitable for various tissue engineering and biomedical applications.