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Naturally derived biofunctional nanofibrous scaffold for skin tissue regeneration

S. Suganya[a,b], J. Venugopal[c], S. Ramakrishna[c], B.S. Lakshmi[b], V.R. Giri Dev[a,*]

[a] Department of Textile Technology, Anna University, Chennai, India
[b] Centre for Biotechnology, Anna University, Chennai, India
[c] Nanoscience and Nanotechnology Initiative, Faculty of Engineering, National University of Singapore, Singapore, Singapore

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ABSTRACT

Significant wound healing activity of Aloe vera (AV) and higher elastic strength of Silk fibroin (SF) along with mammalian cell compatibility makes AV and SF an attractive material for tissue engineering. The purpose of the present work was to combine their unique properties, with the advantage of electrospinning to prepare a hybrid transdermal biomaterial for dermal substitutes. The physico-chemical characterization of the developed scaffold showed finer morphology expressing amino and esteric groups with improved hydrophilic properties and favorable tensile strain of 116% desirable for skin tissue engineering. Their biological response showed favorable fibroblast proliferation compared to control which almost increased linearly by ($p < 0.01$) 34.68% on day 3, ($p < 0.01$) 19.13% on day 6, and ($p < 0.001$) 97.86% on day 9 with higher expression of CMFDA, collagen and F-actin proteins. The obtained results prove that the nanofibrous scaffold with synergistic property of AV and SF would be a potential biomaterial for skin tissue regeneration.

1. Introduction

Skin is the largest protective barrier of human body. Skin regenerate itself when subjected to minor injuries however severe damages like full thickness dermal loss require effective clinical treatment failure of which may lead to mortality [1]. Surgical approaches like auto grafts remains the main treatment for thermal injury related skin loss however severe burn patients lack tissue availability requiring an alternative method of skin replacement [2]. Emergence of tissue engineering therapeutic means has attracted much attention as it offers a better solution to overcome the drawbacks of current limitation in skin transplantation which focuses on regeneration of neotissues from cells with the support of biomaterials and growth factors [3]. The cells, scaffold and growth factor are the three key materials for tissue engineering [4]. Scaffolds are artificial structure capable of supporting and providing a native environment for the cell adhesion and proliferation to form tissues [5,6].

Several ways available for scaffold production of which electrospinning remains the predominant choice as it can produce materials with nanoscale properties [7] mimicking the architecture of the native extracellular matrix [8]. Polymeric scaffold of different origin (natural and synthetic) have been investigated for scaffold development [9,10]. Natural materials like collagen, fibrin and chitosan are rich in growth factors and are ideal for promoting skin tissue regeneration but are not mechanically strong when electrospun on the other hand biodegradable synthetic materials such as Polycaprolactone (PCL), Poly(L-lactic acid) (PLLA) are stronger, but lack growth factor for tissue regeneration [11–13]. The major challenges in scaffold fabrication for dermal regeneration are the need for both complex functionality and biomechanical stability. The alternative solution for the above issue can be overcome by physical hybridizing of polymers (natural biopolymers or synthetic polymers) for a synergistic actions and then converting them into nanofibers which can impart bioactivity and improved mechanical property to the resulting scaffold.

Silk fibroin is a natural protein isolated from silkworm (*Bombyx mori*) containing two main proteins, sericin (outer covering) and fibroin (central structure). Fibroin does not induce immune rejection unlike other bio-derived proteins and hence being focused for biomedical applications. Additionally fibroin has many unique properties like strong mechanical stability; biocompatibility and slow degradability. Research findings have shown

* Corresponding author at: Department of Textile Technology, A.C.Tech, Anna University, Chennai-600 025, Tamilnadu, India. Tel.: +91 44 22359248; Mobile: +91 9486600246.
E-mail addresses: vrgiridev@yahoo.com, vrgiridev@annauniv.edu (V.R.G. Dev).

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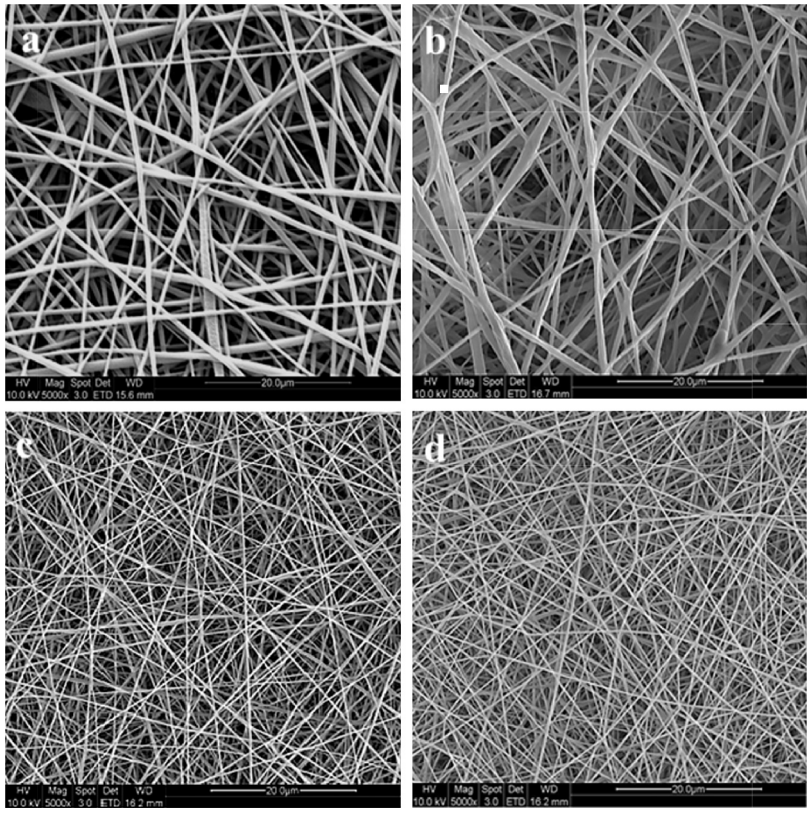

Fig. 1. FESEM morphologies of (a) PLACL, (b) PLACL-SF, (c) PLACL-SF-AV and (d) PLACL/Collagen nanofibrous scaffolds. The average diameter of the composite fibers decreased and the morphology of fibers became finer with the incorporation of Aloe vera.

fibroin based scaffolds mimic the extracellular matrix and efficiently support cell attachment and proliferation of fibroblasts [14–25]. Aloe vera based dermatological treatment have been practised traditionally for centuries especially for wounds, burns, insect sting, and skin inflammation [26–28]. Anti-oxidant, anti-inflammatory, anti-microbial, immunomodulatory properties of aloe vera has highlighted its application in biomedical applications. Mannose 6-phosphate and acemannan are the important components responsible for many therapeutic properties of Aloe vera which mediate cell signaling pathway for proliferation of fibroblast [29,30]. It promotes epithelialization and collagen synthesis for effective wound healing [31–34].

Human fibroblast cells need higher elastic support due to their elongated morphology. In present study for scaffold fabrication PLACL was used which has higher tensile properties compared to PCL and Silk Fibroin was used to further increase the mechanical strength of the fabricated scaffolds as silk fibroin has unique mechanical and biological properties and Aloe vera was used for its wound healing property. Physico-chemical and biological characterization of the hybrid scaffolds are investigated for its enhanced ability towards wound healing and tissue regeneration.

2. Materials and methods

2.1. Fabrication of PLACL, PLACL-SF, PLACL-SF-AV and PLACL/Collagen scaffolds

PLACL was dissolved in DCM: DMF (3:1) (Sigma-Aldrich, St. Louis, USA) to form 10% solution and kept in stirring overnight. PLACL-SF solutions were prepared by dissolving 8% PLACL and 4% lyophilized Silk Fibroin powder (Xi'an Yuensun Biological Technology Co., Ltd, China) in DCM: DMF (3:1). Similarly PLACL-SF-AV solution was prepared by dissolving 8% PLACL followed by addition of 4% silk fibroin powder and 4% Aloe vera powder (Xi'an Yuensun Biological Technology Co., Ltd, China) in DCM:DMF (3:1). PLACL/Collagen solution was prepared by dissolving 8% PLACL and 2% collagen in 10 ml HFP. The solutions were fed into a 5 ml standard syringe attached to a 21G blunted stainless steel needle, respectively, using a syringe pump (KDS 100, KD Scientific, Holliston, MA) at a flow rate of 0.75 ml/h with an applied voltage of 17 kV for all the solutions (Gamma High Voltage Research, USA). Random fibers were collected on a flat collector plate wrapped with aluminum foil that was kept at a distance of 12–13 cm from the needle tip. On application of high voltage the polymer solution was drawn into fibers. These nanofibers were collected on 15 mm cover slips by spreading them on the collector plate and used for cell culture studies.

2.2. Characterization of nanofibrous scaffolds

The electrospun nanofibers were sputter-coated with gold (JEOL JFC-1200 Fine Coater, Japan) and visualized using a field emission scanning electron microscope (FESEM) (JEOL JSM 6700, Japan). Diameters of the electrospun fibers were analyzed from the SEM images using image analysis software (Image J, National Institutes of Health, USA). FTIR spectroscopic analysis of electrospun nanofibrous scaffolds was performed on Avatar 380 (Thermo Nicolet, Waltham, MA, USA) over a range of 500–4000 cm^{-1} at a resolution of 2 cm^{-1}. Tensile properties of electrospun nanofibrous scaffolds were determined with a tabletop tensile tester (Instron 3345, USA) using load cell of 10 N capacities. Rectangular specimens of dimensions 10 mm × 20 mm were used for testing, at a crosshead speed of 10 mm/min and the data was recorded for every 50 microseconds. Tensile stress strain and elastic modulus were calculated based on the generated tensile stress–strain curve. Hydrophilic nature of the electrospun nanofibrous scaffolds were measured by sessile