Abstract

Skin graft is an essential treatment module for patients with major skin loss to recover from acute or chronic wounds. When affected area is exceeding 80% of the body surface, unavailability of healthy skin limits autografting; further, repeated harvesting may create scarring, pain and delayed healing of donor site. Additionally, use of allografts is also limited as they often undergo immunogenic rejection. Development of an alternative readily available bioengineered skin analog stimulating wound bed for faster and improved healing would be beneficial. Mimicking three-dimensional organization of the skin, the present work aims to develop a bilayer skin substitute comprising a porous 3D nanofibrous layer with ECM resemblance underneath a dense nanofibrous membrane which would limit cellular infiltration and benefit compartmentalization.

A simple and differential methodology of polycaprolactone (PCL) -chitosan emulsion preparation resulted in development of bilayered scaffold with overlying nanofibrous membrane of PCL-chitosan on underlying cotton-like fluffy 3D nanofibrous PCL-chitosan layer coated with collagen. The electrospun membrane had fiber diameter ~ 274 nm and pore size ~ 1.16 µm while fluffy 3D layer had fiber diameter ~ 1.62 µm and pore size ~ 20.59 µm. The 3D layer was further coated with collagen I isolated from mrigal fish scale to improve bio functionality. Surface coating with collagen I resulted in bundling of fibers together, thereby increasing their average diameter to 2.80 µm and decreasing pore size to ~ 15.34 µm. Distinct pore size variation created a bilayered structure resembling dermal extracellular matrix.

The architecture and composition of the scaffold promoted efficient cellular activity where high interconnected porosity with ECM resembling collagen I coating assisted in cellular adhesion, infiltration and proliferation from initial days of fibroblast seeding while keratinocytes grew as monolayer on the dense nanofibrous membrane. Anatomy of the scaffold arising due to variation in pore size is promising in compartmentalization and preventing initial cellular transmigration. The scaffold supported extracellular matrix protein expression and stratified epithelialization *in vitro* developing human skin equivalent model. Effective integration and attachment of scaffold with margins of a third degree wound created in a rat model and accelerated healing in comparison to control proved its utility as skin regenerating scaffold. Simple technique with inexpensive raw materials endorsed the scaffold as a promising off-the-shelf cost-effective matrix for skin tissue engineering.

Keywords: Bilayered porous 3D scaffold, Emulsion electrospinning, Skin tissue engineering, Burn wound healing