Abstract

Large full-thickness skin defects resulting from acute/chronic wounds or trauma represent a major unsolved clinical problem. Despite the fact that bioengineered skin and their application on human patients have become a reality, scientists and surgeons are still exploring to develop an optimal therapeutic approach that would replace today's gold standards. The present research investigates role of a bilayer nano/micro fibrous chitosan-collagen based dermal rudiment, in facilitating healing of full-thickness skin lesions.

In this study, sequential electrospinning and freeze-drying process was employed for development of bilayer scaffolds with hierarchical nano/microfiber architecture. Primarily, a chitosan-PEO blend solution was electrospun to produce a nanofiber mat having ~78 nm fiber diameter and nanoscale inter-fiber spacing. While doing so, effect of viscosity and yield stress on chitosan based nanofiber fabrication was clearly evidenced. Architectural stability of nanofibers in aqueous medium was achieved by tripolyphosphate mediated ionotropic cross-linking of chitosan. 3T3 fibroblast cells showed good response on nanofiber mats thereby indicating matrices' cytocompatible nature. For development of bilayer scaffold, collagen type I (pH ~6) solution was freeze-dried over pre-fabricated chitosan based nanofibers. Scaffolds thus developed comprised of an upper chitosan based nanofiber layer with nano-scale pore sizes, and a collagen fiber based sublayer with multiscale fiber diameter and pore sizes. Physico-chemical properties of scaffolds, including swelling, biodegradation, mechanical properties were found suitable for intended application. 3T3 fibroblasts and HaCaT keratinocytes cultured on scaffolds showed appreciable cellular response. Attempts were made to co-culture 3T3 and HaCaT on scaffolds so as to develop a reconstructed skin model. To asses the full-thickness wound healing potential, scaffolds were tested in an *ex vivo* human skin equivalent wound model, as a preliminary alternative to animal testing. The results showed keratinocyte migration and wound re-epithelisation, but limited fibroblast infiltration- a condition that was attributed to instability of scaffold stacks in full-thickness lesions. To overcome this limitation, three dimensional (3D) plug scaffolds (1-2 mm thick) were prepared by increasing thickness of collagen sublayer. 3D scaffolds unveiled significant cellular responses *in vitro* and induced insignificant hemorrhagic effect. When applied onto full-thickness skin lesions *in vivo*, scaffolds integrated with wound bed/margin, thereby facilitating easy cellular infiltration, migration, re-epithelisation, while preventing contraction and scarring. Taken together, the results suggest that herein proposed chitosan-collagen scaffolds show significant potential as a dermal rudiment and may evolve as a suitable matrix for skin tissue engineering.

Keywords: Bilayer, Nano/microfiber, Chitosan, Collagen, Skin Tissue Engineering