Abstract

The loss of tissue functions due to diseases, trauma, and injury represents a serious issue in healthcare. Current traditional clinical treatments are often disappointing to restore tissue functions in critical defects. Tissue engineering proposes a new therapeutic avenue in regenerative medicine. The present work aims to develop a functional construct using biomaterials with stem cells, growth factors, or drugs for replacing/restoring the functions of damaged tissue. In this endeavour, monounsaturated fatty acid group functionalized chitosan, i.e. oleoyl chitosan (OC) has been synthesized, characterized with FTIR, ^{13}C NMR, and was used to explore its potential towards bone and skin tissue restoration. Owing to free amine group, this polymer could be incorporated with gelatin and decellularized extracellular matrix to fabricate various kinds of bioactive grafts, which could promote the regeneration of the damaged tissue.

Skin grafting is an indispensable treatment module for large full-thickness skin defects. Bioengineered skin analog comprising resemblance with native extracellular matrix could stimulate augmented wound healing. Nanofibrous mats were fabricated with gelatin and OC, which offered tunable wettability, degradation, and superior biocompatibility. Further, gelatin/OC based nanofibrous mats with diameter of ~ 200 nm, incorporated with amniotic membrane derived stem cells (HAMSCs) implanted in full-thickness wounds demonstrated accelerated skin regeneration in 15 days. Also, OC and dermal decellularized extracellular matrix (dECM) derived thermo-sensitive biohybrid hydrogels were fabricated by self-assembling. The nano-topographical surface of the hybrid hydrogel (diameter 74.6 ± 10.1 nm) and biological cues of dECM encourage cellular behaviour. The hybrid hydrogels exhibited suitable gel strength (G′ ~ 1280 Pa) and stability and superior biocompatibility, accelerated fibroblasts/keratinocytes migration, and evidenced with excellent neo-vascularization proficiency. Also, subcutaneous implantation of this hydrogel did not trigger any inflammation or immune response to the host. The hybrid hydrogels facilitated rapid wound closure in rat model with formation of neo-epithelium and enhanced collagen deposition in dermal layer.

Biohybrid hydrogels was also developed with OC and bone decellularized extracellular matrix (DBM) by self-assembling and crosslinked with genipin. The

nanofibrous structure (diameter 70.9 ± 13.01 nm) and the cytokines or growth factors preserved in the resultant hydrogel mimic native tissue microenvironment, thus providing excellent encapsulation potential of HAMSCs and superior osteogenic differentiation ability. Further, DBM/OC hydrogels with and without HAMSCs implanted in rabbit tibial defects demonstrated enhanced bone regeneration (~ 83%) compared to empty defects. Also, alendronate loaded gelatin microspheres were incorporated in this hybrid hydrogel and bone morphogenic protein-2 was loaded on the hydrogel construct. Hydrogel constructs with both alendronate and bone morphogenic protein-2 showed excellent bone healing potential with mineralized bone matrix after 8 weeks in a rabbit model.

Keywords: Oleoyl chitosan; Decellularized extracellular matrix; Nanofibrillar architecture; Full-thickness wound healing; Bone tissue regeneration; *In vivo* studies.