Abstract

This work used silk fibroin from the Indian tropical tasar silkworm, *Antheraea mylitta*, to develop potential biomaterials for bone tissue engineering. This fibroin is mechanically robust. Its built-in integrin binding peptide RGD (Arg-Gly-Asp) sequences enhance cellular interaction and proliferation, making it osteoconductive and osteoinductive. The fibroin was blended either with poly-vinyl alcohol (PVA) or poly(∈-caprolactone) (PCL) synthetic polymer to electrospin nanofibrous extracellular matrix(ECM). Characterization and comparative *in vitro* studies between 2% silk fibroin (SF)-PVA and 2% SF-PCL blended matrices showed that 2% SF-PCL matrices served as better ECM and it was chosen for subsequent studies.

Surface modification, one of the most promising methods to enhance the cell adhesion and proliferation on hydrophobic surface, was adapted to investigate which incorporation method, blending or grafting, of SF into PCL nanofibrous matrix was better for bone tissue regeneration. Surface of PCL nanofibrous matrices was modified by aminolysis to graft 2% SF and hence comparative *in vitro* and *in vivo* studies were carried out between 2% SF blended and grafted matrices. Results implied 2% SF grafted PCL nanofibrous matrix was superior as ECM for bone tissue regeneration. Hence, this matrix was chosen as ECM for mineralization and bio-active molecule incorporation.

The SF grafted PCL nanofibrous matrix was mineralized by (a) electrodeposition of nHAp (b) alternatively soaking in calcium and phosphate solution and (c) incorporation of nHAp into polymer solution before electrospinning. Bone morphogenic protein-2 (rhBMP-2) and transforming growth factor-beta (TGF-β), independently and in combination, were coupled with the mineralized scaffolds using carbodiimide coupling reaction at physiological concentrations. TGF-β and rhBMP-2 have distinct roles during natural regeneration of bone. Their combined delivery led to significant improvement of osteogenic growth for all mineralized scaffolds. Dual growth factor loaded scaffold from each mineralization process, were compared to determine the optimal mineralization method. Appraisal of *in vitro* osteogenic differentiation of human mesenchymal stem cells, scaffolds' immune response based on cytokine levels in co-culture of human macrophages and osteoblast like cells, and the scaffolds' *in vivo* bone regeneration ability was conducted. The scaffolds were implanted in rabbit distal femur bone defects for 3 months. Efficacy of the scaffolds in repairing critical sized femur defects *in vivo* was determined based on immuno-compatibility, radiography, micro computed tomography, histology, and mechanical push-out. Fibroin grafted PCL nanofibrous scaffolds, mineralized using electrodeposition, was most advantageous in all aspects: *in vitro* osteoconductivity and osseointegration and *in vivo* bone regeneration.

Our work shows that dual growth factor incorporated, silk fibroin grafted, PCL

nanofibrous scaffold mineralized by electrodeposition, was most advantageous for bone tissue reconstruction.

Keywords: Bone tissue engineering, non-mulberry silk fibroin, mineralization, nanofibrous scaffolds, growth factors.