Development of Biocompatible Trilayered Nanocomposite Hydrogel Scaffold for Periodontal Regeneration

Abstract

Periodontitis is a debilitating oral disease that affects the tooth supporting structures namely cementum, periodontal ligament (PDL) and the alveolar bone. The current clinical management entails the removal of the etiological factors specifically plaque and calculus from the tooth surfaces by scaling, root planing and curettage. This non-surgical management is followed by surgical management that includes resective surgery and regeneration of lost tissues by utilization of grafts, GTR membranes or combination therapy. Though the prognosis of the disease has been improved by removal of the causative factors and regeneration of one or more periodontal tissues has been achieved to some extent, most of these techniques have failed to simultaneously regenerate and restore the functionality of the lost or damaged periodontal tissues. To overcome the current limitations, a tri-layered scaffolding approach was adopted to facilitate complete and simultaneous regeneration of hard tissues - cementum, alveolar bone; and soft tissue - the PDL, at a periodontal defect site. The tri-layered nanocomposite hydrogel scaffold was composed of Chitin-PLGA/nBGC/CEMP1 as the cementum layer, Chitin-PLGA/FGF2 as the PDL layer and Chitin-PLGA/nBGC/PRP as the alveolar bone layer. Bioglass nanoparticles (nBGC) were synthesized by sol-gel route and characterized. Chitin-PLGA and Chitin-PLGA/nBGC hydrogels were prepared and lyophilized to obtain Chitin-PLGA and Chitin-PLGA/nBGC hydrogel scaffolds. Growth factors namely recombinant human cementum protein 1 (rhCEMP1) into Chitin-PLGA/nBGC, recombinant human fibroblast growth factor 2 (rhFGF2) into Chitin-PLGA and platelet rich plasma derived growth factors (PRP) into Chitin-PLGA/nBGC hydrogel scaffolds were incorporated. The prepared hydrogel scaffolds were porous in nature. In addition, possessed swelling ability, biodegradability, mechanical property and favored protein adsorption. All the growth factors showed an initial rapid release followed by sustained release up to 14 days. For the *in vitro* culture, dental follicle stem cells (DFCs) were isolated from human dental follicle and

characterized. The isolated hDFCs were mesenchymal in origin. These cells also expressed PDL and cementum specific markers namely periodontal ligament attachment protein (PLAP1), CEMP1 and FGF2 which confirmed the existence of progenitor/precursor populations in the dental follicle tissue. Further, the hDFCs were subjected to cementogenic, fibrogenic and osteogenic differentiation in the presence of specific growth factors and induction medium. CEMP1, BSP, COL1 and OPN expressions confirmed the cementogenic differentiation. FGF2, PLAP1 and COL1 expressions confirmed the fibrogenic differentiation. RUNX2, ALP, matrix mineralization, COL1 and OPN expressions confirmed the osteogenic differentiation. Gene expression also validated the differentiation of hDFCs. The tri-layered nanocomposite scaffold supported the viability, adhesion and proliferation of hDFCs followed by cementogenic, fibrogenic and osteogenic differentiation. *In vivo*, maxillary periodontal defects were created in rabbit models. The tri-layered nanocomposite hydrogel scaffold with/without growth factors were implanted and compared with positive and negative controls. Defect closure and periodontal regeneration was analyzed at 1 and 3 months post-operatively through 3D micro-CT reconstruction, histopathology and immunohistochemistry. The tri-layered nanocomposite hydrogel scaffold with/without growth factors showed complete defect closure and healing with new cancellous-like tissue formation on Micro-CT analysis. Histological and immunohistochemical analysis further confirmed the formation of new cementum, fibrous PDL and alveolar bone with well defined bony trabeculae. From the results obtained, the tri-layered nanocomposite hydrogel scaffold was found to be an alternative regenerative approach that can be applied to achieve simultaneous and complete periodontal regeneration.