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The Role of a Biphasic Implant Based on a Bioactive Silk Fibroin for Osteochondral Tissue Regeneration

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Abstract : Biphasic scaffolds in cartilage tissue engineering have been designed to influence not only the recapitulation of the osteochondral architecture but also to take advantage of the healing ability of bone to promote the implant integration with the surrounding tissue and then bone restoration and cartilage regeneration. This study reports the development and characterization of a biphasic scaffold based on the assembly of a cartilage phase constituted by fibroin biofunctionalized with bovine cartilage matrix; cellularized with differentiated pre-chondrocytes from adipose tissue stem cells (autologous) and well attached to a bone phase (bone bovine decellularized) to mimic the structure of the nature of native tissue and to promote the cartilage regeneration in a model of joint damage in pigs. Biphasic scaffolds were assembled by fibroin crystallization with methanol. The histological and ultrastructural architectures were evaluated by optical and scanning electron microscopy respectively. Mechanical tests were conducted to evaluate Young's modulus of the implant. For the biological evaluation, pre-chondrocytes were loaded onto the scaffolds and cellular adhesion, proliferation, and gene expression analysis of cartilage extracellular matrix components was performed. The scaffolds that were cellularized and matured for 10 days were implanted into critical 3 mm in diameter and 9-mm in depth osteochondral defects in a porcine model (n=4). Three treatments were applied per knee: Group 1: monophasic cellular scaffold (MS) (single chondral phase), group 2: biphasic scaffold, cellularized only in the chondral phase (BS1), group 3: BS cellularized in both bone and chondral phases (BS2). Simultaneously, a control without treatment was evaluated. After 4 weeks of surgery, integration and regeneration tissues were analyzed by x-rays, histology and immunohistochemistry evaluation. The mechanical assessment showed that the acellular biphasic composites exhibited Young's modulus of 805.01 kPa similar to native cartilage (400-800 kPa). In vitro biological studies revealed the chondroinductive ability of the biphasic implant, evidenced by an increase in sulfated glycosaminoglycan (GAGs) and type II collagen, both secreted by the chondrocytes cultured on the scaffold during 28 days. No evidence of adverse or inflammatory reactions was observed in the in vivo trial; however, In group 1, the defects were not reconstructed. In group 2 and 3 a good integration of the implant with the surrounding tissue was observed. Defects in group 2 were fulfilled by hyaline cartilage and normal bone. Group 3 defects showed fibrous repair tissue. In conclusion; our findings demonstrated the efficacy of biphasic and bioactive scaffold based on silk fibroin, which entwined chondroinductive features and biomechanical capability with appropriate integration with the surrounding tissue, representing a promising alternative for osteochondral tissue-engineering applications.

Keywords : biphasic scaffold, extracellular cartilage matrix, silk fibroin, osteochondral tissue engineering
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