Acta Biomaterialia 8 (2012) 3283–3293

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Simultaneous regeneration of articular cartilage and subchondral bone induced by spatially presented TGF-beta and BMP-4 in a bilayer affinity binding system

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ARTICLE INFO

Article history:
Received 19 January 2012
Received in revised form 9 May 2012
Accepted 14 May 2012
Available online 19 May 2012

Keywords:
Affinity-binding scaffold
Bilayer
TGF-β1
BMP-4
Subchondral defect

ABSTRACT

Subchondral defect repair is a multitask challenge requiring the simultaneous regeneration of cartilage and bone. Herein, we describe the features of a hydrogel system designed to simultaneously induce the endogenous regeneration of hyaline cartilage and subchondral bone. The system was constructed as two layers, spatially presenting the chondroinductive transforming growth factor-β1 (TGF-β1) in one layer and the osteoinductive bone morphogenetic protein-4 (BMP-4) in a second layer, via affinity binding to the matrix. Human mesenchymal stem cells seeded in the bilayer system differentiated into chondrocytes and osteoblasts in the respective layers, confirming the spatial presentation and prolonged activity of TGF-β1 and BMP-4. Administration of the bilayer system with affinity-bound TGF-β1 and BMP-4 (with no cells) into a subchondral defect in rabbits induced endogenous regeneration of articular cartilage and the subchondral bone underneath within 4 weeks. Cartilage extracellular matrix proteoglycans were found in the top layer, with no mineralization, whereas the layer underneath consisted of newly formed woven bone. The results indicate that stem cells migrating into the defect are able to sense the biological cues spatially presented in the hydrogel and respond by differentiation into the appropriate cell lineage. The strategy has a real translational potential for repairing osteochondral defects in humans as it is acellular and can be implanted via a minimally invasive method.

1. Introduction

A subchondral defect is usually characterized by a structural breakdown of the articular cartilage and the bone underneath due to trauma or disease, leading to chronic disabilities. Treating such defects is an important target in regenerative medicine since damaged cartilage cannot spontaneously heal itself in adults. The avascular cartilage, with its dense extracellular matrix, prevents chondroprogenitors from migrating to the injury site, thus greatly reducing the tissue's regenerative potential. For decades, cell-based strategies have been developed for treating cartilage defects, involving the injection of cell suspensions (chondrocytes, stem cells) or their combination with biomaterials [1,2]. The strategies have encountered crucial barriers in therapeutic translation, due to concerns with the cells in use (e.g. rejection, pathogen contaminants, tumorigenesis) and technical issues (packaging, storage, shipping), as well as difficulties in clinical adoption and regulatory approval [3].

Recently, following the identification and efficient production of molecular inducers of tissue regeneration and with the development of hydrogels for their sustained delivery, there has been a shift in trend towards adopting acellular therapeutic approaches. Such strategies have been fueled by the finding that microfracture surgical techniques can induce the recruitment of bone marrow stem cells into the chondral defect and initiate its repair [4]. To enhance endogenous cell recruitment, combinations of molecular inducers and biomaterials have been applied to prolong factor activity [5,6]. These strategies for repairing osteochondral defects are still in their infancy, and in need of additional testing [7,8].

Herein, we aimed to examine whether the complex task of osteochondral regeneration can be achieved by administrating the chondroinductive transforming growth factor-β1 (TGF-β1) [9] and the osteoinductive bone morphogenetic protein-4 (BMP-4) [10–12], presented spatially in two distinct hydrogel layers. We hypothesized that such a hydrogel system would attract host stem cells to differentiate in situ into either chondrocytes or bone cells, depending on the layer to which they migrate. To enhance the local activities of the factors, TGF-β1 and BMP-4 were attached to the matrix hydrogel by including alginate-sulfate, a synthetic biomaterial synthesized in our laboratory that has been shown to affinity-bind heparin-binding proteins [13].

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http://dx.doi.org/10.1016/j.actbio.2012.05.014