Design of a Biphasic Scaffold Exhibiting Two Inductive Cues for Reconstructing the Osteochondral Interface

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Introduction: Human MSC differentiation depends on the environment wherein the cells reside, especially on the spatio-temporal presentation of the differentiation-inductive factors. Herein, we aim to reconstruct the microenvironment promoting the osteochondral differentiation of MSCs, by presenting the chondro-inductive Transforming Growth Factor-β1 (TGFβ1), and the osteo-inductive Bone Morphogenetic protein-4 (BMP-4) in a biphasic alginate scaffold, in a similar manner to their presentation by the extracellular matrix. Thus, TGFβ1 and BMP4 were affinity-bound to alginate-sulfate containing scaffold, mimicking the specific interactions of this factor with heparan sulfate. [1]

Methods: TGFβ1 or BMP4 affinity-bound to two separate alginate-sulfate/alginate scaffolds were prepared by a freeze-dry technique (200ng protein/scaffold). The released TGFβ1/ BMP4 from the scaffolds were analyzed by ELISAs and their bioactivity by measuring collagen deposition in a fibroblast culture. HMSC were seeded into the TGFβ1 or BMP4/affinity-bound scaffolds (300,000 cells/scaffold), and the GF-induced signal-transduction pathways were tested by Western Blot analysis. Within TGFβ1/affinity-bound scaffolds, collagen deposition in hMSC constructs was detected by Masson's trichrome staining, and type II collagen was detected by immuno-staining. Osteogenic differentiation in BMP-4 scaffolds was assessed by alkaline phosphatase activity (ALP).

Results: A sustained factor release for 7 days was seen from the TGF-β or the BMP-4 affinity-binding scaffolds, in contrast to the burst factor release from the unmodified alginate scaffolds. TGFβ1/BMP4 retained their activity, as assessed by the enhanced collagen deposition in the fibroblast culture. Human MSCs, seeded in these scaffolds, showed prolonged and enhanced expression of phosphorylated Smad2 and ERK1/2, for up to 14 days, indicating the long-term activity of the affinity-bound factors **(Fig.1)**. Masson's trichrome staining and immuno-staining of 14 days-old cell constructs demonstrated massive deposition of collagen and collagen type II, a marker for cartilage ECM, in the section of the TGFβ1/affinity-bound scaffolds. The cells presented round morphology of committed chondrocytes **(Fig.2A)**. In the BMP-4/affinity-bound scaffold section, elevated secretion levels of alkaline phosphatase were found, indicating hMSC differentiation into the osteo-lineage cells **(Fig.2B)**.

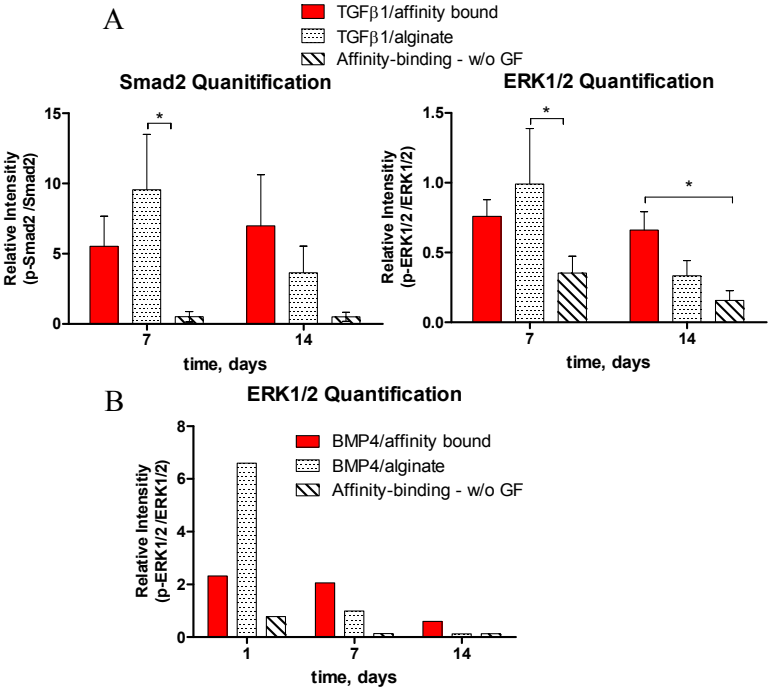

Fig.1 TGFb1-induced (A) and BMP4-induced (B) signaling pathways in hMSC cell constructs. Densitometric analysis of Western blots. * - $p<0.05$.

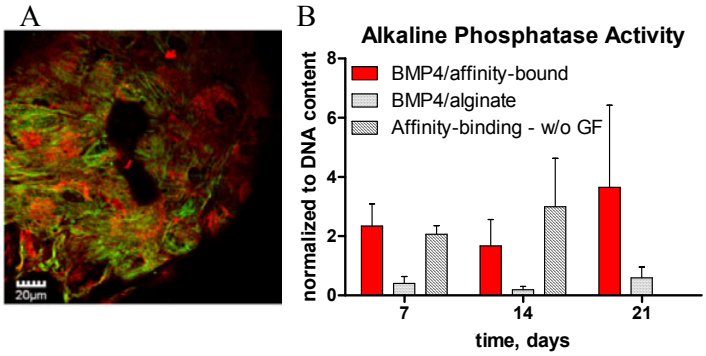

Fig.2 Induced differentiation of hMSC. Immuno-staining for vimentin (green) and collagen II (red) in TGFβ1/affinity bound scaffolds (bar= 20μm) (A). Prolonged alkaline phosphatase activity in BMP4/affinity bound scaffolds for 3 weeks of cultivation (B).

Conclusions: These data indicate the potential use of the affinity-binding alginate scaffolds combined with spatial presentation of TGFβ1 and BMP4 for reconstruction of the microenvironment inducing osteochondral interface.

References: 1. Freeman, I, Kedem, A, Cohen, S. (2008) Biomaterials, **29**, 3260.