IGF-I time-concentration profiles can modulate the early stage differentiation of human mesenchymal stem cells: application to ligament or cartilage engineering

Marcos Garcia-Fuentes, Lorenz Meinel, Hans P. Merkle

Drug formulation and delivery group, Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093 Zurich, Switzerland

INTRODUCTION: Traumatic injuries of musculoskeletal tissues pose a significant problem to current medicinal practise. Mesenchymal stem cells (MSCs) are self-replicating multipotent cells with the capacity to regenerate structural and connective tissues of the body when in the presence of suitable environmental cues [1]. Nevertheless, the biochemical pathways that regulate MSC differentiation are mostly unknown, and, therefore, engineered grafts cannot yet match the properties of native tissues. In this work we hypothesized that insulin-like growth factor-I (IGF-I) is an important signal in MSC differentiation, and that commitment of MSCs to specific cell lineages can depend on the concentration of this cytokine present at different stages of cell differentiation.

METHODS: Silk fibroin was purified and porous scaffolds from this biomaterial were prepared as previously described [2]. MSCs were seeded onto the scaffolds and cultured in DMEM supplemented with fetal bovine serum, ascorbic acid, non-essential amino acids, dexamethasone (10 nM) and TGF-β1 (1 ng/ml) (control medium). Total culture time was 3 weeks and medium was changed every other day. IGF-I was added to the control medium in three regimens: 0 ng/ml for the whole experiment (regimen 0, control), 50 ng/ml for the whole experiment (regimen 1), and 100 ng/ml for first week and 25 ng/ml for the two further ones (regimen 2); thus regimens 1 and 2 had the same total amount of IGF-I supplemented during the whole culture time. Cell medium for the test samples was also changed every other day during the experiment, but IGF-I was supplemented every day. Tissues were analyzed for differences in cell proliferation (DNA measurement), glyco-aminoglycan content, gene expression (Real Time RT-PCR) and histology (hematoxylin-eosin staining).

RESULTS: All engineered tissues displayed an structured fibrous structure typical for ligament-like grafts. Histological analysis confirmed that culture of MSCs resulted in fibroblastic morphology. IGF-I treated constructs showed enhanced proliferation and enhanced extracellular matrix formation.

Gene expression analysis of the samples showed that the main structural protein of ligaments (collagen type-I) was up-regulated for all of the experimental groups and controls. We also found that MSCs cultured in the presence of IGF-I can overexpress typical makers of cartilage such as collagen type-II and SOX9, but this occurred for an intermediate stage in cell development only. Intriguingly, by changing the IGF-I dosing regimen, we could also shut down collagen type-II and SOX9 expression. Under the test II dosing regimen (i.e. the one that concentrates most of the IGF-I dose at the initial culture time points), collagen type-II expression remained at levels similar to those of reached by samples without IGF-I.

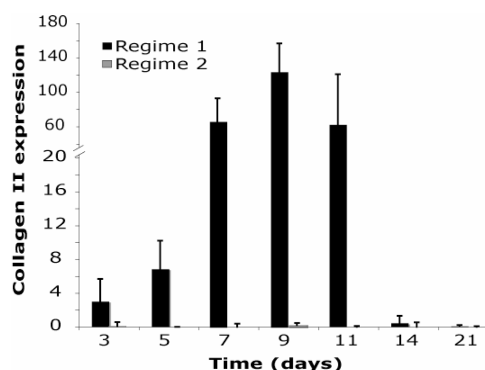

Fig. 2: Gene expression levels of collagen type-II in MSCs cultured over a 21 period (mean ± SD, n=6). Expression levels were normalized to the housekeeping gene GAPDH and are relative to those of non-cultured MSCs (day 0).

DISCUSSION & CONCLUSIONS: IGF-I appears to be deeply involved in both cartilage and ligament development. Particularly, IGF-I concentration in the first stages of MSC differentiation might be an important regulator in lineage commitment.

REFERENCES: [1]S Hofmann et al., Tissue Eng 2006; [2]S Sofia et al., J Biomed Mater Res 2001.

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