Understanding cellular behaviour in early and late stage of MSD

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Introduction

Osteoarthritis (OA) is the most common joint disease worldwide with only limited treatment options[1]. It is believed that the thorough analyses of the disease on cellular and molecular base can lead us to the discovery of new targets, and to the development of new therapies. Thus, we have been working on the development of a 3D model of human OA knee cartilage. By this *in vitro* model, it is expected to gain a better understanding on the disease process. The two main aspects of our research are: (1) how the mechanical loading and (2) how the presence of pro-inflammatory cytokines, e.g. TNF-α and IL1-β affects the degenerative processes[2,3]. In the present study, we demonstrated the effect of the different cytokines on monolayer and tissue culture.

Materials and Methods

All human cartilage samples were obtained from the local hospital. The cartilage pieces were harvested from the lateral-central regions of both the femurs. The biopsy specimens as well as the cell monolayers were cultured in tissue culture polystyrene (TCPS) multi-well plates, immersed in Dulbecco's modified Eagle's medium (DMEM) (Sigma – Aldrich, USA) with phenol red, supplemented with 10% foetal bovine serum (FBS) (Biochrone, Berlin, Germany) 1% antibiotic-antimycotic solution (Invitrogen, USA) and cytokines TNF-α, and IL1-β (R&D systems – Minneapolis, USA) and incubated for 6 days at 37°C, 5% CO_2, within static conditions (ATM 0.1MPA). The cellular viability was assessed by Live/Dead® Viability Assay (C3099, P1304MP, Invitrogen). Cell metabolic activity was evaluated by MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-rboxymethoxyphenyl)-2(4-sulfofenyl)-2H-tetrazolium) assay (G3581, CellTiter 96® AQueous Assay, Madison, WI, USA). GAG content of the cartilage biopsy specimens was assessed by Safranin O staining.

Results

The cell morphology of the chondrocytes was altered as a consequence of cytokine treatment. Instead of the roundish phenotype elongated fibroblastic cells were observed after 6 days of incubation with cytokines (Fig.1). Cell viability showed lower rates when cytokine was added in the culture media as it is shown by the Live/Dead assays, however the MTS assay indicated an increasing cell metabolic activity in the presence of cytokines. The tissue culture showed accelerated degradation processes and increased GAG loss when the media was supplemented with cytokines as it was shown by Safranin O and Fast green staining on the histological images.

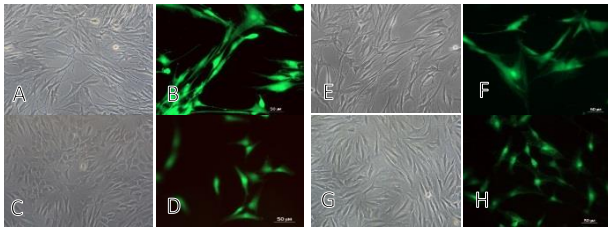

Figure 1. Cell viability - Live/Dead assay. A – B, healthy chondrocytes, 25ng/ml IL-1ß, 100 ng/ml TNF-α; E – F: OA chondrocytes 25ng/ml IL-1ß, 100 ng/ml TNF-α; C – D: control, healthy chondrocytes, G – F: control, OA chondrocytes.

Conclusion

The performed studies showed that the cytokines lead the chondrocytes towards the osteoarthritic phenotype and accelerate the degradation processes in the cartilage tissue as it was expected, therefore ideal agents for the development of the envisioned 3D OA cartilage model. However, further studies are needed to evaluate the effects of cytokines on molecular and gene level.

Acknowledgement

This study was performed by the foundation of FP7 Marie Curie Initial Training Network "MultiScaleHuman": Multi-scale Biological Modalities for Physiological Human Articulation (2011-2015), contract MRTN-CT-2011-289897.

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