The improvement of paracrine activity of multipotent mesenchymal stromal cells in 3D culture conditions

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INTRODUCTION

The multipotent mesenchymal stromal cells (MSCs) are extensively studied for use in cell therapy and regenerative medicine due to their significant therapeutic potential mostly attributed to their paracrine activity [1]. MSCs *in vivo* reside in a tissue-specific 3D microenvironment that ensures their stemness and potential preservation. However, large-scale expansion of MSCs *in vitro* in a highly oxygenated 2D environment affects cell metabolic status, proliferation, differentiation, growth factor/cytokine secretion, and induces cell senescence [2]. Transfer of MSCs into 3D culture conditions that mimic the natural microenvironment can restore cellular properties and is an affordable strategy for cell pre-treatment before therapy [2]. However, no comparative research has been reported to determine the optimal 3D culture conditions for enhancing the paracrine activity of MSCs.

MATERIALS AND METHODS

Adipose tissue MSCs from different donors from passages 4-5 (N=3 in duplicates). We applied several 3D culture conditions: a) spheroid cultures without ECM, b) hydrogel cultures supported by ECM (collagen, plasma-based hydrogel, Geltrex™), c) porous Geltrex-based scaffolds and d) 2D conditions as a control. We prepared collagen hydrogels for embedding MSCs from Collagen I (rat tail, 3 mg/ml) according to manufacturer's instructions. Plasma-based hydrogels were prepared using human plasma, human serum and $CaCl_2$. Geltrex™ is a soluble form of basement membrane matrix with reduced content of growth factors. Growth factors production was assessed using the 11-Plex Human ProcartaPlex assay followed by measurement on a Bio-Rad Luminex instrument. The metabolic activity of the cells was determined using the alamarBlue and Seahorse XF assays. Cell viability in 3D cultures was assessed by the LIVE/DEAD™ assay and subsequently evaluated on a spinning disk confocal microscope.

RESULTS AND DISCUSSION

In this study, we evaluated the production of selected growth factors by MSCs cultured in different 3D culture conditions. We found, that in most cases, 3D culture conditions significantly increased growth factor production by MCSs and changed their metabolic activity. The amount of growth factors produced was affected by the applied different 3D culture microenvironments and varied depending on the presence of extracellular matrix, origin and structural composition.

CONCLUSIONS

We found that the paracrine activity of MSCs is significantly affected by the 3D culture microenvironment. Advances in understanding the relationships between the microenvironment and paracrine activity may be the key to achieving successful and effective MSC-based clinical therapies.

ETHICAL STATEMENTS

All human tissue donors provided their written informed consent before any intervention. All studies involving human tissues or cells were approved by the Ethics Committee of the Institute of Experimental Medicine of the CAS, Prague, Czech Republic. All methods were performed in accordance with the relevant ethical guidelines and regulations.

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