Two year long-term study of human MACT-grafts *in vitro*

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INTRODUCTION: Matrix-associated autologous chondrocyte transplantation (MACT) has already been broadly investigated but remains poorly understood concerning the biological reasons for the variability of clinical results. Due to the restricted availability of repair tissue of patients, long-term cultures were performed. The aim of this study was to investigate tissue formation, differentiation of human articular chondrocytes under artificial conditions and degradation of the scaffold material.

METHODS: Grafts of human articular chondrocytes cultivated on the collagen I/III scaffold Chondrogide® (MACI®) were taken 1) at the time of transplantation (three days after seeding) 2) three weeks, 3) three months and 4) two years after *in vitro* culture. Investigations were performed using light, scanning and transmission electron microscope and *in situ* hybridization.

RESULTS: At the time of transplantation, grafts of the patients were very different concerning cell number and cell morphology. During the following cultivation time cell-scaffold constructs with little infiltration but a differentiating tissue at the scaffold surface developed. The differentiation proceeded in a cell multi-layer underneath an outer fibroblast layer simultaneously with the secretion of extracellular matrix (ECM). First signs of differentiation, such as spherical cells containing round or lobed nuclei, few cytoskeletal elements, glycogen accumulations, lipid droplets and further dense ECM consisting of flocculent and fibrous elements (Fig.1), appeared after three weeks of cultivation. *In situ* hybridisation confirmed the differentiated stage of the cells by the presence of collagen type II mRNA. After two years of *in vitro* cultivation a hyaline-like tissue has been formed (Fig.1). Chondrocytes infiltrated the whole scaffold but without the formation of larger cell populations. During the first month of cultivation, some of the transplants formed roulades with the cell population in the middle (Fig.1). The biodegradable fleece showed obvious dissociation of the large, looser fibres; the small fibres remained morphologically unaffected during the whole cultivation time.

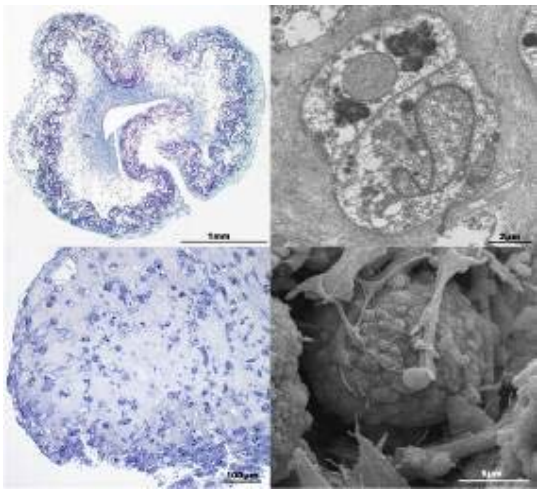

Fig. 1: Chondrocyte grafts after long-term cultivation: 'Roulade' of transplant (upper left); differentiating chondrocyte (upper, right), hyaline-like tissue (lower left), spherical chondrocyte in hyaline-like tissue (lower right).

DISCUSSION & CONCLUSIONS: During the long term cultivation of the MACI®-grafts a hyaline-like tissue developed without supplementary stimuli, such as mechanical stimulation or additional factors (e.g.: growth factors). The differentiating cell populations were located on the fleece surface suggesting only few or hardly any influence of the scaffold material. Differentiation proceeded simultaneously with an increase of autogen ECM, which is probably responsible for the phenotype switch. Contractile properties of the superficial cells of the cell layer are thought to affect the curving of the transplant and the formation of 'roulades'.

ACKNOWLEDGEMENTS: This study was supported in part by the Lorenz-Boehler-Society (10/04). We thank the institution 'Cell Imaging and Ultrastructural Research' for the possibility to use of the electron microscopic equipment.