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We previously showed that high hydrostatic pressure (HHP) treatment at 200 MPa for 10 min induced complete cell death in skin and skin tumors via necrosis. We used this technique to treat a giant congenital melanocytic nevus and reused the inactivated nevus tissue as a dermis autograft. However, skin inactivated by HHP promoted inflammation in a preclinical study using a porcine model. Therefore, in the present study, we explored the pressurization conditions that induce apoptosis of the skin, as apoptotic cells are not believed to promote inflammation, so the engraftment of inactivated skin should be improved. Using a human dermal fibroblast cell line in suspension culture, we found that HHP at 50 MPa for ≥ 36 h completely induced fibroblast cell death via apoptosis based on the morphological changes in transmission electron microscopy, reactive oxygen species elevation, caspase activation and phosphatidylserine membrane translocation. Furthermore, immunohistochemistry with terminal deoxynucleotidyl transferase dUTP nick-end labeling and cleaved caspase-3 showed most cells in the skin inactivated by pressurization to be apoptotic. Consequently, in vivo grafting of apoptosis-induced inactivated skin resulted in successful engraftment and greater dermal cellular density and macrophage infiltration than our existing method. Our finding supports an alternative approach to hydrostatic pressure application.

Abstract 83

IL-17 CONTROL OF CHONDROCYTE NUCLEAR AND CELL SHAPE AND FUNCTION: POSSIBLE IMPACT ON CARTILAGE REPAIR MECHANISMS

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IL-17 has been implicated in cartilage degradation and inflammation. We aimed to understand how inflammation, cell morphology and cell function are linked. Articular cartilage superficial zone explants and chondrocytes from patients having different grades of macroscopic osteoarthritic (OA) degeneration were examined to determine a) whether there was a differential response of isolated chondrocytes to IL-1 β vs. IL-17A and b) if OA cartilage explants responded similarly. Explants were exposed to injury and inflammatory cytokines, while chondrocytes were exposed to inflammatory cytokines. IL-1 β caused a significant decrease in COL2A1 gene expression in both tissue explants and chondrocytes. IL-17A also resulted in a significant decrease in chondrocyte COL2A1 expression. In tissue explants, COL2A1 showed a decreasing trend in response to IL-17 and the decrease in expression was similar to IL-1 β treatment. In contrast, in comparison to IL-1 β , IL-17A resulted in a significantly decreased inflammatory response (IL-8 gene expression) in both chondrocytes (59-fold decrease, 24h; 10-fold decrease, day 7) and OA explants (32-fold decrease, 48h). Despite a decreased IL-8 mRNA expression response, IL-17A caused high secretion of soluble IL-8 from chondrocytes which correlated dose-dependently with nuclear size and shape. In chondrocytes, COL1A2 expression was not significantly altered by IL-17A, but its expression significantly correlated with cell aspect ratio, roundness, circularity, and solidity and nuclear aspect ratio and roundness. Importantly, IL-17A dose-dependently decreased COL2A1 expression which correlated

with chondrocyte roundness and nuclear aspect ratio and roundness. Therefore, IL-17A is capable of controlling cell and nuclear shape and chondrogenic cell function which may impact cartilage repair.

Keywords: Inflammation; Cell shape; IL-17

Abstract 84

3D MODELLING STRATEGIES TO PREDICT THE MECHANICAL BEHAVIOUR OF POLYMERIC SCAFFOLDS BY ADDITIVE MANUFACTURING

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Additive Manufacturing (AM) is a well known method to produce scaffolds with different geometrical patterns (grid, honeycomb, gyroid, etc.). These hierarchical porous structures can be designed ad hoc to enable specific mechanical properties and functionalities to provide cell viability and tissue regeneration. In particular, the AM technology based on material extrusion (MEX, commonly known as FDM) produces 3D porous structures processing several thermoplastic biopolymers. Nevertheless, the operational parameters and the characteristics of the deposition process generate a part in which real geometry is different from the theoretical one of the 3D model. This difference involves an additional problem for simulating the mechanical behaviour of the scaffold, for example by Finite Element Analysis (FEA). The paper presents two developed methods to predict the real geometry of scaffolds made by MEX and starting from G-code files. The first method is the "automated sweep CAD modeller of extrusion-based G-code (DECODE)" and the second the "volume conserving model for 3D printing (VOLCO)". DECODE reads the G-code and generates several scripts to automate the 3D CAD modelling (sweep features) to reproduce the extrusion paths. VOLCO builds a voxel-based model starting from the G-code and considering some features in the deposition process. Both methods were compared in terms of volume of the resulting geometry of the scaffolds, and the FEA simulation and testing of the stiffness. DECODE obtains more accuracy in volume (compared to the theoretical models) and can manage larger parts, while VOLCO, after a fitting process, is more accurate for mechanical prediction by FEM.

Abstract 85

SOLUTIONS FOR REPOPULATION OF DENSE CARTILAGE MATRIXES.

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Decellularised organs and tissues are promising scaffolds for tissue regeneration providing the accurate composition and architecture to support targeted tissue regeneration. In this process, repopulation of devitalized matrixes is the most critical and challenging step, especially in dense tissues such as cartilage. To overcome this difficulty, several chemical and mechanical strategies have been developed. Articular cartilage can be enzymatically extracted from specific matrix components such as elastin, creating channels accessible for cells to grow into the dense matrix. However, for articular cartilage chemical treatment for selective removal of matrix