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PROTEIN FIBRILS AS SCAFFOLD MATERIAL FOR CARTILAGE TISSUE ENGINEERING: EFFECTS ON CELL VIABILITY AND PROLIFERATION

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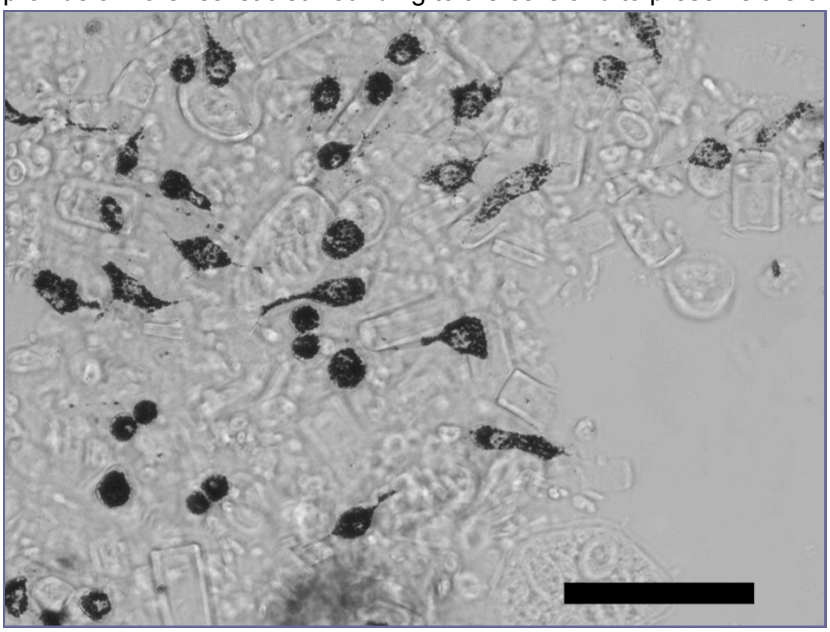
Abstract:
Purpose:
Current treatment options for osteoarthritis (OA) aim to reduce pain and improve joint functioning. However, focal cartilage damage due to trauma predisposes the onset of OA. Use of scaffolds is a good strategy for the treatment of these small lesions. In the past, various synthetic and natural proteins have been described for their use as scaffolding material for tissue engineering. However, all of these scaffolds have advantages and disadvantages in terms of the biological compatibility, strength, elasticity and durability of the material. The perfect scaffolding material for articular cartilage has not been found. We propose a new strategy for generation of cartilage scaffold material.
Many proteins and peptides can self-assemble into β-sheet rich amyloid fibrils. The resulting fibrils are up to tens of nanometres wide and several micrometres in length, and can cluster into large aggregates. As scaffolding material, these amyloid fibrils have interesting characteristics for the generation of cartilage tissue: their mechanical properties are comparable to collagen fibres, they are degradation resistant, they form hydrogels and can be functionalized. The purpose of this study was to characterize amyloid fibrils and to investigate the effect of amyloid structures on bovine chondrocyte viability and metabolic activity.
Methods:
The proteins α-synuclein, β-lactoglobulin and lysozyme self-assembled into amyloid fibrils. Their formation and resulting structures were analysed using circular dichroism, Thioflavin T fluorescence, atomic force microscopy (AFM) and scanning electron microscopy (SEM). Bovine articular cartilage was collected from bovine knees and chondrocytes were isolated from the cartilage using collagenase 2. The bovine chondrocytes were cultured in monolayer and in the presence of amyloid fibrils or aggregates (Figure 1). The viability of the chondrocytes was analysed using Calcein AM in combination with flow-cytometry and the bulk metabolic activity was investigated using MTT. Changes in gene expression, among others cartilage specific genes like SOX9, COL2A1 and ACAN, in the presence of amyloid structures were analysed using qPCR. In addition, the effect of the amyloid fibrils on cell viability and metabolism was investigated using different amyloid concentrations and incubation times.
Results:
SEM imaging confirmed the formation of fibrils. AFM imaging confirmed the formation of fibrils and showed different morphologies and heights depending on the different proteins used to form amyloid fibrils. Results indicate that the presence of α-synuclein amyloid aggregates has no negative effects on cell viability or metabolic activity after one week in culture. Cartilage marker gene expression went down after culturing for one week in presence or absence of amyloid aggregates, indicating that the presence of amyloid aggregates did not have an effect on the cartilage gene specific gene expression. Finally, bovine chondrocyte proliferation appears to increase in the presence of the aggregates.
Conclusions:
The results indicate that the different proteins can form amyloid fibrils with different morphologies and heights. In addition, α-

synuclein amyloid aggregates do not appear to influence cell viability and cartilage gene expression. The gradual loss of articular cartilage genes was probably due to dedifferentiation of the chondrocytes in a monolayer culture. The effect of morphological differences between the fibrils and aggregates generated from different proteins on the cell viability and metabolic activity will be investigated in more detail. The results indicate that the amyloid structures could potentially be used for cartilage tissue engineering. The next steps include culturing chondrocytes with amyloid structures in a 3D hydrogel to provide a more realistic surrounding to the cells and to preserve the chondrocyte phenotype.



Microscopy image of bovine chondrocytes (dark) in culture with α-synuclein amyloid aggregates (transparent). The dark color of the cells is caused by formazan crystals as a result of the MTT assay. Scale bar represent 100 µm.

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