differentiation was confirmed by an increase in type 1 collagen, osteocalcin and osteopontin transcript expression and Alizarin red positive staining. An increased expression of type II collagen and aggrecan transcripts and a positive alcian blue staining was finally observed in the presence of a chondrogenic differentiation medium. Interestingly, the expression of transcripts coding for NPCy specific markers including OVOS2, Pax1 and CA12 were increased upon stimulation with a nucleopulpogenic differentiation medium and confirmed by immunohistochemistry. Finally our data also indicate that we were able to transplant about 400µl of Si-HPMC by percutaneous intradiscal injection in several lumbar IVD.

Conclusions: Our data have first confirmed the stemness properties of sheep adipose-derived stromal cells by demonstrating their proliferation, clonegenicity and multipotency. In the context of IVD regenerative medicine, it was of particular interest to show that sheep ASC were able to give rise to nucleopulpocytes when cultured in an inductive medium. Finally, our results also highlight our ability to inject a large volume of SI-HPMC in sheep IVD, compatible with future preclinical tests. In this context, sheep could be considered as a preclinically relevant animal model for comparing IVD regenerative strategies. Further studies dedicated to evaluate the regeneration of NP niche through biomaterial-assisted transplantation of sASC are now under investigation.

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LYSOZYME SELF-ASSEMBLES INTO AMYLOID NETWORKS THAT SUPPORT CARTILAGE TISSUE FORMATION

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Purpose: No cure is available for repair of damaged cartilage. Once damaged, cartilage will continue to degenerate, resulting in immobile and painful joints. Treatments are limited to symptom relief, while tissue engineering approaches fail to produce cartilage comparable to native articular cartilage in terms of strength and modulus.

In this project, we investigated the use of self-assembling proteins as scaffold material for cartilage tissue engineering. These so-called amyloid networks consist of amyloid fibrils forming physical cross-links. The fibrils self-assemble from proteins by forming inter-protein β-sheets. Amyloid networks resemble the extracellular matrix of cartilage since the strength and Young's modulus of the fibrils is comparable to those of collagen and the networks are hydrogels. We therefore hypothesized that amyloid networks can be used as scaffold material for cartilage tissue engineering.

Methods: The proteins α-synuclein, β-lactoglobulin and lysozyme were incubated under self-assembly conditions. Formation of amyloid fibrils was confirmed by using the amyloid-specific fluorescent dye ThT. The change in secondary structure was measured with CD-spectroscopy and the morphology of the obtained structures was visualized with electron microscopy. The formation of amyloid networks was confirmed with electron microscopy and ThT.

Bovine chondrocytes were isolated from calf knees using collagenase and used at passage 1. The cells were cultured in monolayer for 3 days in de presence of monomers or amyloid networks of the 3 proteins to study their effect on chondrocyte viability. The percentage healthy chondrocytes was quantified with the Calcein-AM dye and flow cytometry. The metabolic activity was measured with MTT assays. The relative change in gene expression was studied with qPCR for anabolic, catabolic and hypertrophy marker genes.

Amyloid networks were mixed with chondrocytes and cultured in 3D for 5 weeks to investigate whether the networks allow cartilage extracellular matrix formation. Samples were sectioned and stained histologically for aggrecan and collagen type 2.

Results: A-synuclein, β-lactoglobulin and lysozyme self-assembled into amyloid fibrils as confirmed by the fluorescent signal of ThT, the change of their natural secondary structure to a β-sheet rich conformation, and their fibrous morphology. These fibrils formed amyloid networks that remained ThT positive.

These networks influenced the viability of bovine chondrocytes. The percentage healthy cells increased significantly in presence of amyloid networks as compared to the monomers or when no additional proteins were added, while the metabolic activity decreased significantly in the presence of amyloid networks. All amyloid networks had a limited effect on the expression of COL2A1 and ACAN as compared to controls without amyloid networks. A-synuclein and β-lactoglobulin networks increased the expression of MMP1 and MMP13.

After 5 weeks of culture, chondrocytes produced aggrecan when cultured in agarose gels. A previously used hydrogel for cartilage formation, RADA-16, was used as a positive control. Chondrocytes cultured in RADA-16 produced almost no aggrecan. A limited amount of aggrecan was produced in the α-synuclein and β-lactoglobulin amyloid network samples. Lysozyme networks increased aggrecan production compared to agarose gels.

Conclusions: Amyloid networks are best known for their presence in several pathologies. However, these structures are used throughout nature as a functional biomaterial, also in humans. Here we show that several proteins can form amyloid networks and that these networks have a positive effect on chondrocyte viability and the formation of cartilage extracellular matrix when cultured in 3D. These results indicate that amyloid networks can be used as scaffold material for cartilage tissue engineering. Furthermore, the amyloid networks are easy to produce, perform better than the synthetic hydrogel RADA-16, and there appears to be a protein specific effect.

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RESURFACING OF DAMAGED ARTICULAR CARTILAGE SURFACES USING ARTHROSCOPIC AIRBRUSHING

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Purpose: There is large unmet need for regenerative treatment of eroded articular cartilage surfaces in end stage osteoarthritis. Arthroscopic airbrushing of chondrocytes or mesenchymal stromal cells (mcs) in an appropriate biomaterial is a promising approach to resurface the joint surface. Recently we have provided proof of concept that arthroscopic airbrushing can be used to fill up focal chondral defects with fibrin glue mixed with chondrocytes and mcss. However, while high-viability results have been reported, occasional cell death hampers widespread introduction of this method. In particular, the influence of the cell type, the biomaterial, airbrushing parameters, and the surface properties on cell viability is still poorly understood, and therefore this problem cannot yet be solved. Thus, the development of a treatment using airbrushing could strongly benefit from a clear understanding of the influence of the process parameters on cell survival, which is the aim of the current work.

Methods: Here we present experimental results and a basic theoretical model describing the cell viability as a function of the cell-surrounding droplet, and impact surface characteristics. The model connects (1) the cell survival as a function of the membrane elongation, (2) the membrane elongation as a function of the cell-containing droplet size, velocity, and viscosity, and (3) the substrate properties (stiffness). The model input parameters are experimentally quantified, using ultra-high-speed, high-resolution photography of airbrush droplet populations at a range of spraying conditions. The model is validated by cell-viability measurements. In these experiments, the droplet size, impact velocity, viscosity, nozzle-substrate distance and substrate stiffness were varied.

Results: Improved cell survival is observed by reducing the surrounding liquid's viscosity, reducing the droplet impact velocity, increasing the droplet size and increasing nozzle-substrate distance. Impact on soft tissues dampens cell deformation during impact, also improving cell viability. The analytical cell survival model is validated by these results, which supports the herein proposed mechanisms for cell deformation and subsequent cell survival or death.

Conclusions: In conclusion, we physically model cell deformation during droplet impact and show how cell deposition parameters can be adapted to increase cell viability. This approach allows for rational optimization of arthroscopic airbrush devices by controlling the droplet characteristics and surface properties. In particular, we expect that airbrush devices with a monodisperse droplet size (instead of the usually employed polydisperse droplet size distribution) will result in improved cell viability in (clinical) applications. We are currently working on such solutions in which droplets of specific size and velocity are created. With this set-up we can look at further optimizations for airbrushing combinations of cells and biomaterials. We will demonstrate recent preliminary results, which already show improved control of cell deformation and viability.