CARTILAGE TISSUE ENGINEERING
A STUDY ON HOW TO IMPROVE CARTILAGE REPAIR

Akademisk avhandling
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This thesis is based on the following papers:

I. Hildner F, Concaro S, Peterbauer A, Wolbank S, Danzer M, Lindahl A, Gatenholm P, Redl H, van Griensven M.,
Human adipose-derived stem cells contribute to chondrogenesis in coculture with human articular chondrocytes.
Tissue Eng Part A. 2009 Dec; 15(12):3961-9.

II. Concaro S, Nicklasson E, Ellowsson L, Lindahl A, Brittberg M, Gatenholm P.
Effect of cell seeding concentration on the quality of tissue engineered constructs loaded with adult human articular chondrocytes
J Tissue Eng Regen Med. 2008 Jan;2(1):14-21.

III. Stenhamre H*, Concaro S*, Brantsing C, Enochson L, Gatenholm P, Lindahl A, Brittberg M.
The in vivo chondrogenic potential of chondrocytes seeded in hyaluronic acid based scaffold is triggered by the degree of redifferentiation in vitro.
* These authors contributed equally and should both be considered first authors.
Submitted after revision to Cells, Tissues and Organs.

IV. Concaro S, Concaro C, Brantsing C, Lindahl A and Brittberg M.
How to improve the in vivo chondrogenic properties of chondrocyte seeded scaffolds; A study on the effect of different nutrition media compositions and culture time.
Submitted after revison to Tissue Engineering.

V. Concaro S, Lönnqvist C, Gatenholm P , Lindahl A and Brittberg M.
A study on how different biomimetic material properties influence the proliferation and migration capacity of porcine articular chondrocytes.
Submitted.

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ABSTRACT

One of the first examples of musculoskeletal tissue engineering is autologous chondrocyte implantation (ACI).The first patient with a cartilage lesion was operated with ACI in 1987 and at that time suspension implantation was used. Today, we use the third generation of ACI where scaffolds are employed to support redifferentiation and neocartilage formation *in vitro* and further maturation *in vivo* after implantation to treat the cartilage defects.

A great deal of information is still needed to clinically improve cartilage production. Variables such us the cell seeding density, the cell culture media formulation, the degree of redifferentiation and the material and biological properties of the scaffold used remain to be investigated further.

In the work reported in paper I we aimed to elucidate whether mesenchymal stem cells (MSC:s) are better than committed chondrocytes in producing cartilage in vitro, whether the co-culture of MSC:s and chondrocytes play a role in enhancing cartilage production in vitro and if different biomaterials affect the differentiation capacity *in vitro*.

The effect of the cell seeding concentration was evaluated in paper 2 by culturing human adult chondrocytes in chitosan scaffolds. After 14 and 28 days in a 3D culture, the constructs were assessed for collagen, glycosaminoglycans and DNA content. The mechanical properties of the constructs were determined using a dynamic oscillatory shear test.

In paper III we studied whether the degree of redifferentiation of chondrocytes in *in vitro* cultured scaffolds had an effect on the neocartilage formation after implantation. It was studied whether redifferentiation of the chondrocytes was accomplished by recapitulating the signaling pattern used by chondrocytes during fetal development.

In paper IV we tried to determine the effect of different culture conditions on the *in vivo* chondrogenic capacity and integration properties of human tissue engineered chondrocyte constructs.

In paper V we evaluated the biomimetic properties of different materials. Materials with good biomimetic properties may influence the initial phases of tissue regeneration by inducing a strong migration of cells into the pores of the scaffold.

Materials and Methods

MSC:s and human adult and pig chondrocytes were cultured in different materials in order to prove the different hypotheses. The chondrocyte differentiation *in vitro* and *in vivo* was evaluated using real time PCR to asses the expression of different genes. The total amount of collagen and proteglycans was determined biochemically. Inmunohistochemistry and different histological scores were used to evaluate the presence of cartilage specific proteins and to semiquantify the histological aspect of tissue engineered constructs after in vitro or in vivo evaluation. A novel transmigration assay was designed to evaluate the biomimetic properties of different biomaterials. To evaluate the in vivo chondrogenic potential, tissue engineered constructs produced *in vitro* were subcutaneously implanted in nude mice or into cartilage defects in human osteochondral plugs.

Results

Related to the number of chondrocytes used, coculture with MSC:s led to a strong increase in collagen type IX mRNA expression, an indicator for long-term stability of cartilage. Chondrocytes had better redifferentiation potential as compared to MSC:s. Tissue glue Tisseel® provided slightly better chondrogenic conditions than Tissue Fleece®.

We determined that concentrations of 12–25 million cells/cm^3 are needed in a chitosan scaffold to increase the matrix production and mechanical properties of human adult chondrocytes under static conditions.

We were able to determine that the *in vitro* chondrogenesis in scaffolds induce a signalling pattern similar to the one seen in fetal development. Furthermore the results indicates that redifferentiation of *in vitro* expanded articular chondrocytes is needed at the time of implantation for neocartilage formation. However, 14 days of preculture *in vitro* used clinically today might be reduced.

Conclusion

It is possible to significantly improve cartilage repair by using the right amount of cell concentration in seeded scaffolds, chondrogenic cells co-cultured and by choosing the right type of biomimetic scaffolding material. The future of cartilage repair lies in further development of suitable materials and good quality cells expanded under the most ideal conditions.

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