Purpose of visit

Osteoarthritis is one of the most important joint diseases with a high prevalence worldwide[1]. Unfortunately, the intrinsic repair capacity of articular cartilage is limited. At the moment, there is no therapy available altering the pathobiologic course of the disease. Therefore, the prognosis for patients suffering from OA is relatively poor[2].
Many attempts are currently made to improve OA therapy. The aim of regenerative medicine is to regenerate damage or even loss of hyaline cartilage. A promising approach to reach this aim is the use of adult pluripotent mesenchymal stem cells (MSC) due to their chondrogenic differentiation potential. MSC can be harvested from autologous patient material like bone marrow or fat tissue[3, 4].
For OA treatment the cells can be applied to the joint by intra-articular (i.a.) injection.
They can be applied to an intra-articular (i.a.) defect site either in a supportive biomatrix to target a focal defect, or by i.a. injection. The i.a. injection of autologous MSC is a low invasive, safe, and cost efficient procedure and therefore a promising treatment for OA patients.
Not only animal studies showed improvement of joint structures and symptoms after i.a. MSC therapy in OA models[5, 6]. Recently a clinical trial in 4 OA patients examined the effect of i.a. MSC transplantation. Summarizing, three out of four patients showed amelioration of clinical symptoms. The authors concluded that the results were encouraging but not excellent and that an enhanced technique may even improve the results[7].
A possible key factor for enhancing this therapy is to improve the attachment of injected MSC to the damaged cartilage. The ability of MSC to adhere to articular cartilage is discussed controversially in the literature. On the one hand a study in a goat model of OA showed attachment of injected MSC to joint capsule and ligaments, but not to hyaline cartilage[8]. On the other hand animal studies proved the adhesion of a small number of injected, labeled MSC to damaged cartilage. In these studies the cells integrated with the surrounding tissue and were embedded in newly formed matrix[5, 6]. The beneficial effect as well as the amount of cells adhering to articular cartilage correlated with the applied cell number[9].
Therefore, we hypothesized that the improvement of cell adhesion to damaged cartilage would result in better tissue regeneration due to increased matrix formation.
Previous data obtained in our laboratory suggest that the adhesion of MSC to articular cartilage might be dependent on the application vehicle (unpublished data). Improved cell attachment to osteoarthritic cartilage by modification of the application vehicle could improve OA therapy in a simple and low-invasive way.
To investigate the factors governing the adhesion of MSC to healthy and damaged cartilage we developed an *in vitro* test system for quantifying cell attachment to osteochondral explants in a rat model. Labeled MSC were seeded on explants obtained from rat tibia plateaus. Adhering cells could be stained and quantified. We tested different vehicles like PBS, hyaluronan, plasma, serum or ion solutions for cell application. Significant differences in cell adhesion to the cartilage surface were observed between the groups.
Before performing any *in vivo* experiments in rat or mouse models, we planned to confirm these *in vitro* results from our rodent systems in a human system as humans are the most important target species for OA therapy. The use of human material gives the most significant insights in this field of study.
For this purpose I spent three months in the lab of Dr. Gerjo van Osch at the Erasmus medical center in Rotterdam (the Netherlands). Her lab is one of the best orthopedic research labs in Europe. At the Erasmus University it was not only

possible to get the necessary human material for our work; Dr. Gerjo van Osch is one of the leading experts for osteoarthritis research and cartilage biology. With the expert knowledge of this group on the culture of human MSC (hMSC) as well as on cartilage biology and pathologic processes during OA development we aimed to

- set up an *in vitro* test system for quantifying MSC attachment to human osteoarthritic cartilage and to
- *study the influence of various soluble factors on the attachment of MSC to osteoarthritic articular cartilage*

Description of the work carried out

The first part of the visit was used to create an *in vitro* test system for quantification of hMSC attachment to human OA cartilage.
Cartilage was obtained from patients undergoing total knee replacement. Discs of 6mm diameter were harvested and seeded with bone marrow derived hMSC. The cells were used in low passages (p2-p4). Prior to seeding they were labeled with superparamagnetic iron oxide particles (SPIO) for tracking purposes as described previously[10]. After an incubation period the discs were washed to remove non-adhering cells and fixed in 4% formalin. The attached MSC were stained with Perl's iron stain according to the manufacturer's protocol. Pictures in 40-fold magnification were taken with an inverse light microscope, and the area covered by stained cells was quantified using ImageJ software. Results were confirmed by histologic examination of frozen cartilage sections.
In a first set of experiments the test system was standardized by defining an optimal seeding density for hMSC and doing a time course study on cell adhesion to the OA cartilage explants.
Using this *in vitro* model the effect of soluble factors on the attachment of hMSC to OA cartilage was examined. With standardized seeding densities and incubation -periods different application vehicles were tested for their influence on cell adhesion to OA cartilage *in vitro*. Taking the results of our rodent model into account physiologic saline solutions containing increasing concentrations of serum were tested and compared to a serum-free control group. In comparison saline solutions containing different concentrations of Mg^{2+} and Ca^{2+} as well as combinations of both ions were examined. Samples were stained and further processed as described above.

Description of the main results obtained

We were able to create a test system for quantifying MSC adhesion to OA cartilage explants *in vitro.* Increasing seeding densities could be detected as an increase of the area covered by labeled cells using ImageJ software. Variations of 50,000 cells in the seeding solution could be clearly detected with this system.
To standardize the model we first selected a standard seeding density that allows us to recognize increased as well as decreased cell numbers adhering to the cartilage surface. A time course experiment was performed to select an optimal incubation time for further experiments. An increase of hMSC adhesion could be seen up to one hour. After this initial period the area covered by labeled hMSC further changed due

to cell spreading on the sample surface. Therefore the optimal incubation time for examining an effect on cell adhesion was defined as one hour.
In our second set of experiments we examined the possible effect of the application vehicle on cell adhesion to OA cartilage. We could detect increasing cell attachment with increasing amounts of serum in the application vehicle. A maximum increase was reached with 50% serum in the seeding solution. Therefore we conclude that serum contains essential factors mediating the adhesion of MSC to cartilage *in vitro*. As one of these factors might be ions present in serum we also tested the effect of increasing Mg^{2+} and Ca^{2+} concentrations in the application vehicle as well as combinations of both. We found that both ions modulated the attachment of hMSC to cartilage.

Future collaboration with the host institute

My visit in Rotterdam was a very educative and productive time. We plan to further collaborate with the group of Dr. G. van Osch. There is a regular exchange of data and ideas with the host institute.

Projected publications/articles resulting from the grant

The work carried out during my visit gave crucial insights concerning the role of the application vehicle on the adhesion capacity of human MSC to cartilage. Taking them into account we now plan to perform in vivo experiments in a rat model to corroborate our in vitro data. In cooperation with the host institute we will write a scientific article on the topic.

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