Gene expression and cell differentiation in matrix-associated chondrocyte transplantation grafts: a comparative study

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SUMMARY

Objective: Although scaffold composition and architecture are considered to be important parameters for tissue engineering, their influence on gene expression and cell differentiation is rarely investigated in scaffolds used for matrix-associated autologous chondrocyte transplantation (MACT). In this study we have therefore comparatively analyzed the gene expression of important chondrogenic markers in four clinically applied cell-graft systems with very different scaffold characteristics.

Methods: Residuals (n = 165) of four different transplant types (MACI©, Hyalograft©C, CaReS® and Novocart©3D) were collected during surgery and analyzed for Col1, Col2, aggrecan, versican, melanoma inhibitory activity (MIA) and IL-1β by real-time PCR. Scaffold and cell morphology were evaluated by histology and electron microscopy.

Results: Despite the cultivation on 3D scaffolds, the cell differentiation on all transplant types didn’t reach the levels of native cartilage. Gene expression highly differed between the transplant types. The highest differentiation of cells (Col2/Col1 ratio) was found in CaReS®, followed by Novocart®3D, Hyalograft®C and MACI©. IL-1β expression also exhibited high differences between the scaffolds showing low expression levels in Novocart®3D and CaReS® and higher expression levels in MACI© and Hyalograft®C.

Conclusions: Our data indicate that scaffold characteristics as well as culture conditions highly influence gene expression in cartilage transplants and that these parameters may have profound impact on the tissue regeneration after MACT.

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Introduction

Lesions of articular cartilage are a large medical-therapeutic problem, as self regeneration potential of cartilage is very limited. Untreated cartilage defects often lead to ongoing pain, negatively affects quality of life and predispose for osteoarthritis in the long term1-3. Therefore articular cartilage injuries are a prime target for regenerative techniques such as tissue engineering.

In contrast with other surgical techniques, which often lead to the formation of fibrous or fibrocartilaginous tissue4, tissue engineering aims to restore the complex structure and properties of native cartilage by the chondrogenic potential of transplanted cells5. Chondrocytes, which are responsible for the production and homeostasis of cartilage extracellular matrix (ECM), are encapsulated by their own matrix and are not available for healing of cartilage defects under natural conditions. To overcome this problem for tissue engineering, chondrocytes are isolated from small biopsies of cartilage taken from a non-weight-bearing area of the joint, propagated in vitro, and implanted into the cartilage defect. During proliferation in monolayer culture, however, the chondrocytes become fibroblast-like and cease the production of Col2, aggrecan and others that account for the specific mechanical properties of cartilage in vivo5-8. In order to obtain a tissue similar to native cartilage this dedifferentiation process has to be reversed. The most common way to induce redifferentiation is to relocate the cells into a 3D environment. This is reached by seeding the cells on different graft systems9,10. Other methods, including the application of low oxygen tension11,12, mechanical stimulation13,14 or the addition of differentiation factors15,16, are also described in literature.

The success of grafts lies in mimicking native structure and in support of cell growth and production of tissue-specific ECM. In the case of a surgical procedure the use of grafts also facilitates the handling and implantation of the cells. The newest generation of the autologous chondrocyte transplantation takes advantages of these
cell seeded grafts and is called matrix-associated autologous chondrocyte transplantation (MACI) [2]. To meet the requirements for the clinical use, a scaffold must be highly Bio-compatible, non-toxic, resorbable and has to fulfil specific mechanical properties — including stability and resilience. A lot of research has been done to develop new biomaterials that can serve as scaffolds and promote chondrogenic differentiation. However, despite the huge variety of available scaffolds, only few of them are in clinical use today. These scaffolds differ highly in their manufacturing process, composition and mechanical properties, and can be classified in natural biomaterials, which are further distinguished as protein-based (collagen, fibrin [8,19] and polysaccharide-based biomaterials (alginate, chitosan, hyaluronic acid, cellulose [20–22]), and in synthetic biomaterials (poly [lactic-co-glycolic acid] (PGA), polyethylene glycol (PEG) [23–25]). Recently, new promising techniques to optimize the adhesion, growth and differentiation of the cells have come into the focus of interest including surface modifications [26–28], coating with bioactive molecules [29] and designed materials [30,31].

However, the influence of scaffold characteristics on the gene expression and differentiation of chondrocytes in clinical applied materials remain poorly investigated. In particular, comparative studies including more than two of those scaffolds are missing. We recently have demonstrated that certain scaffold parameters could influence cell distribution and adhesion [32]. In this study we have focused on the differences in gene expression and differentiation of the cells in four clinical applied scaffolds that exhibit a highly different composition and morphology [21,33–35]. For this purpose 165 transplant samples as well as 26 biopsies were assessed by real-time PCR in terms of their matrix production (Col2, Col1, aggrecan, versican), expression of the differentiation factor melanoma inhibitory activity (MIA) and the pro-inflammatory parameter IL-1β.

Materials and methods

Cartilage and transplant specimens

Residuals of four different types of MACT grafts (n = 165 transplants in 165 patients) were used: Hyalograft C autografts (Fidia Advanced Biomaterials, Italy), a hyaluronan web; MACI (Genzyme, USA, former Verigen, Germany) a collagen type I/III membrane. This collagen membrane is manufactured by Geistlich (Switzerland) and is separately available under the trade name Chondro-Gide [36]. Genzyme (former Verigen, which was bought by Genzyme in 2005) uses this membrane to produce their chondrocyte transplant called MACI in a production facility in Europe. The collagen type I gel CaReS (Arthro Kinetics Biotechnology GmbH; Austria); and Novocart 3D (TeTeC, Germany), which is a bilayered collagen type I sponge containing chondroitin-sulphate.

The grafts are produced by the companies as follows:

Hyalograft C autograft: The patient’s chondrocytes are isolated from biopsies and multiplied in a monolayer culture. According to the company the maximum number of passages for 2D cultivation is defined in the production process, which was not further specified. The cells are thereafter seeded onto the surface of the Hyalograft C web at a density of 1 × 10⁶/cm² and cultivated for at least 2 weeks. The cells are cultivated in foetal calf serum.

MACI: After isolation of chondrocytes from biopsies, the cells are propagated in monolayer and seeded on the rough side of the fleece. Cell number and cultivation time are not exactly given, but according to personal communication, the maximum number of passages is set below the critical number defined by their own data, which was not further specified. 3D cultivation time is about 1 week. The cells are cultivated in autologous serum.

CaReS: As opposed to the three other graft types investigated in this study, the patient’s chondrocytes are mixed with the gel directly after isolation from the biopsies without monolayer cultivation. 3D cultivation is performed for 2 weeks (10–14 days). The cells are cultivated in autologous serum.

Novocart 3D: Patient’s chondrocytes are isolated from full depth cartilage cylinders, multiplied in monolayer without getting passaged and seeded in a density of 1.45 × 10⁶/cm² onto the scaffold. Those constructs are cultivated for about 2 days under 3D conditions before sending the graft to the hospital. The cells are cultivated in homologous serum.

The required amount of cartilage tissue was obtained from biopsies from the femoral intercondylar notch of the knee during arthroscopy and sent to the companies for further processing. A small sample of some biopsies (n = 26) was kept in the hospital for further analysis. Per patient only one biopsy was taken from the affected knee joint and used for analysis. Samples of the cartilage transplants were collected during surgery and stored in the transport medium at room temperature. Immediately after surgery the samples were brought to the laboratory for further processing. The surgeries were all done in the same hospital by one surgeon. Clinical studies were approved by the local ethical board and patient consent was given (147/2003, 148/2003, 420/2003, 307/2006, 738/2010).

RNA extraction and purification

Lysis of the cells was performed by adding 1 ml of TRI Reagent™ (Sigma–Aldrich) to the MACI, Hyalograft C and Novocart 3D scaffold. RNA isolation was performed according to the standard protocol.

For total RNA extraction from native cartilage and the CaReS gel, the samples were frozen in liquid nitrogen and ground using a mortar and pestle. Further steps were performed using the RNeasy® plant mini kit (Qiagen, Germany). The procedure for isolating total RNA was executed as described in the RNeasy® Mini Handbook (Qiagen, Germany, 06/2001).

cDNA synthesis

Total RNA (0.1–1 µg) was diluted with nuclease-free water to a volume of 15 µl. Thereafter 4 µl iScript™ Reaction Mix, as well as 1 µl iScript™ Reverse Transcriptase were added (Bio-Rad Laboratories, California, USA). The reaction mixture was incubated for 5 min at 25°C, for 30 min at 40°C, and for 5 min at 85°C.

Primers and probes for quantitative analyses

Primers and probes were designed using the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and the Primer Express 2.0 software (Applied Biosystems, California, USA), which generates oligonucleotides with similar melting temperatures and minimal self complementarity. To avoid amplification of genomic DNA, the probes were placed at the junction of the two exons. Gene specificity of the primers and probes and the absence of DNA polymorphism were confirmed by BLAST searches. Primers and probes were synthesised by Eurogentec (Seraing, Belgium). Primer concentrations were tested for each primer at concentrations of 50 nM, 300 nM, and 900 nM, choosing the combination that displayed the lowest Ct value. Primer sequences are shown in Table I. For IL-1β the predeveloped Taq Man™ assay (Applied Biosystems, California, USA) was used.

Real-time PCR amplification and analysis

Real-time PCR amplification was performed and monitored using an ABI Prism® 7500 Fast Real-Time PCR System (Applied Biosystems, California, USA). The master mix was based on the
SensiMix™ Probe Kit (Quantace, London, UK). The thermal cycling conditions comprised the initial steps at 50°C for 2 min and at 95°C for 10 min. Amplification of the cDNA products was performed with 40 PCR cycles, consisting of a denaturation step at 95°C for 15 s and an extension step at 60°C for 1 min. Beta-2-microglobulin (B2M) was chosen as the internal standard, using the predeveloped Taq Man® assay (Applied Biosystems, California, USA), since it showed the most consistent expression in our experimental setup and it was already suggested for a similar setup by Foldgard et al.²⁰. For a given amount of cDNA the range of the housekeeping gene was within +/- 1.5 Ct of the median value, samples outside of this range were excluded from analysis. All cDNA samples (2.4 µl in 20 µl) were analyzed in triplicate. The final numeric value was calculated as the ratio of the gene to B2M and was expressed in arbitrary units.

Electron microscopy and histology

Scanning electron microscopic samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. After rinsing with distilled water they were chemically dehydrated with 2, 2-Dimethoxypropane and dried with hexamethyldisilazane. Samples were then sputter-coated with gold and analyzed in a Philips XL 20 scanning electron microscope.

For histological examinations standard protocols were used. In brief, the transplant samples were fixed with 7.5% paraformaldehyde, dehydrated in a series of alcohol, rinsed in xylol and infiltrated with paraffin. Deparaffinized sections were stained with haematoxylin and eosin, alcin blue and fast red or DAPI.

Statistical analysis

All samples were assayed by real-time PCR in triplicates. Statistical evaluation was performed using SPSS software version 17.0 (SPSS Inc., Chicago, USA). Values of the transplant processing time are presented as mean ± standard deviation (SD). Gene expression values are presented as boxplot diagrams. Outliers and extreme outliers were defined as an observation 1.5 and 3.0 × the interquartile range outside the central box, respectively. Normal distribution of the ∆ΔCt values was tested by the Kolmogorov–Smirnov test. Differences between groups were assessed by one-way ANOVA and Scheffé post hoc test and were considered statistically significant when P values were lower than 0.05.

Results

Residuals of 165 cartilage transplants (MACI® n = 22, Hyalograft®C n = 91, CaReS® n = 26 and Novocart®3D n = 26) were collected during surgery and analyzed by real-time PCR. These numbers refer to all data presentations and statistical calculations, except for IL-1β, where they are separately specified. The distribution of patients’ age and sex is shown in Table II.

From 26 patients residuals of the initial biopsy were collected during arthroscopy. These biopsy samples served as control group for native cartilage, in order to analyze the changes occurring during the cultivation process of the different scaffolds. From the biopsies and date of implantation the processing time of each scaffold was calculated. This processing time included the transport, the isolation and the cultivation of the cells until the designated implantation date. The mean values of the processing time were 67 ± 22 days for MACI®, 75 ± 31 days for Hyalograft®C, 15 ± 1 days for CaReS® and 28 ± 6 days for Novocart 3D (Table II).

Scaffold morphology

Scanning electron and light microscopy revealed very different architecture of the four scaffold types (S.E.M. pictures showing scaffolds without cells in Fig. 1). MACI® consist of a dense, fibrous network of small and large fibres with unilateral orientation [Fig. 1(A, C, D)], while Hyalograft®C forms a non woven web of regular fibres with large inter-fibre distances [Fig. 1(E, G, H)]. The collagen gel CaReS® consists of irregular fibres and fine fibrillar material in the interfiber space [Fig. 1(I, K, L)]. Novocart®3D has the most complex architecture with more or less parallel plates interconnected with small fibres [Fig. 1(M, O, P)]. The chondrocytes are unilaterally distributed on the surface of MACI® [Fig. 1(B)] and form a subconfluent to multi-cell layer of mainly elongated cells [Fig. 1(C, D)]. In the three other scaffold types the cells are three-dimensionally distributed [Fig. 1(F, P, J)] but vary in cell number and cell morphology. While Hyalograft®C [Fig. 1(G, H)] and CaReS® [Fig. 1(K, L)] contain a sparse quantity of cells which are either spherical or elongated, most cells with mainly spherical morphology were found in Novocart®3D [Fig. 1(O, P)].

Gene expression analysis

In the biopsies Col1 expression showed a very high variance and no significant differences to the transplant samples, with the exception of Novocart®3D, which expressed Col1 lowest (5.3-fold decrease compared to native cartilage, P < 0.001). The highest Col1 expression of all scaffolds was found in MACI®, the lowest in Novocart®3D (14.3-fold decrease compared to MACI®, P = 0.001). No significant differences were seen between Hyalograft®C and CaReS® [Fig. 2(A)].

A dramatically lower Col2 expression in cells cultured in CaReS vs native cartilage was found [832-fold lower, P < 0.001, Fig. 2(B)]. Although the variance in Col2 expression was very high, especially in Hyalograft®C, the mean values of the different scaffolds were very similar, with the exception of CaReS®, which showed a significantly higher Col2 expression than the others (18-fold, P < 0.001 compared to Hyalograft®C and Novocart®3D).

Very similar results were found for aggrecan [Fig. 2(D)], whose expression decreased during the cultivation (5-fold decrease, P = 0.001, CaReS® compared to native cartilage). Like Col2, aggrecan was expressed significantly more in CaReS® than in the other

| Table I  |
| Description of the designed primers and probes |
| mRNA template | Primer sequence |
| Col1a1 | left: 5′-atgctgctgaaagtggct-3′ |
| | right: 5′-aggagagcattgcactc-3′ |
| | probe: 5′-accacaccccttctgccatc-3′ |
| Col2a1 | left: 5′-gecggctgtctggtggtctc-3′ |
| | right: 5′-gccgccgacagtccgact-3′ |
| | probe: 5′-aaagttgccgaaagctcctg-3′ |
| versican | left: 5′-tcpaggacgacggagcctc-3′ |
| | right: 5′-tcpagggagtcctctgtaga-3′ |
| | probe: 5′-aaggctgtctctgcttcagc-3′ |
| MIA | left: 5′-ggagagccagtctctctgtaa-3′ |
| | right: 5′-gctgagctgcacctgcagatg-3′ |
| | probe: 5′-tcctcatttgtctgtccttc-3′ |

| Table II  |
| Descriptive statistics |
| Scaffold | n | Age at biopsy | Male/female | Processing time (days) | Biopsies |
| MACI® | 22 | 33.64 ± 7.33 | 18/4 | 66.55 ± 21.77 | 3 |
| Hyalograft®C | 91 | 33.91 ± 8.91 | 61/30 | 74.78 ± 31.25 | 18 |
| CaReS® | 26 | 29.27 ± 8.42 | 16/10 | 14.69 ± 1.49 | 5 |
| Novocart®3D | 26 | 29.81 ± 8.07 | 20/6 | 27.96 ± 5.84 | 0 |
| Total | 165 | 32.46 ± 8.67 | 115/50 | 26 |
transplants (13-fold, \( P < 0.001 \), compared to MACI\(^{C210}\)). Plotting Col2 vs aggrecan expression revealed a high correlation between these two genes in all transplant and biopsy samples [Fig. 4(A)].

The large fibroblast proteoglycan versican was expressed lowest in Novocart\(^{C210}\) \( 3D \) [Fig. 2(C)], followed by MACI\(^{C210}\) (1.6-fold, \( P = 0.495 \), compared to Novocart\(^{C210}\) \( 3D \)) and the other two scaffolds (both 3-fold, \( P < 0.001 \), compared to Novocart\(^{C210}\) \( 3D \)). No significant difference between the biopsies and the transplant samples, except for Novocart\(^{C210}\) \( 3D \) (2.5-fold decrease, \( P = 0.005 \), compared to native cartilage), were observed for versican.

MIA or cartilage-derived retinoic acid sensitive protein (CD-RAP) is a protein expressed in developing and mature cartilage. Since its expression is required for cartilage differentiation and depends directly on the differentiation status of the cells\(^{37,38}\), we have chosen MIA as a further differentiation marker for chondrocytes. A good correlation between MIA and the chondrogenic phenotype marker Col2 was demonstrated, when plotting Col2 vs MIA expression of all transplant and biopsy samples [Fig. 4(B)]. MIA expression was found to be significantly reduced in the transplant samples compared to native cartilage (5.9-fold decrease, \( P = 0.005 \), CaReS\(^{C210}\) compared to native cartilage) [Fig. 3(A)]. Among the transplants CaReS\(^{C210}\) and Hyalograft\(^{C}\) exhibited a significantly higher expression of MIA than MACI\(^{C210}\) and Novocart\(^{C210}\) \( 3D \) (7-fold, \( P < 0.001 \)).

Due to the very low expression level of IL-1\( \beta \) in non-osteoarthritic chondrocytes, IL-1\( \beta \) was detected only in six biopsy samples showing no significant differences between the biopsy and the transplants [Fig. 3(B)]. Out of 25 CaReS\(^{C210}\) samples only five were positive for IL-1\( \beta \) suggesting a very low expression in this type of scaffold. However, no statistical significance was observed owing to the low number of positive sample. Novocart\(^{C210}\) \( 3D \) (n = 324) showed also low levels of IL-1\( \beta \) compared to MACI\(^{C210}\) (n = 16, 33.3-fold decrease, \( P < 0.001 \) and
Hyalograft®C (n = 69, 20-fold decrease, P < 0.001). Both, IL-1β and MIA expression, varied highest in Hyalograft®C.

In accordance with Col2 and aggrecan expression, the two calculated ratios (Col2/Col1 and aggrecan/versican) fell dramatically in the transplant samples compared to native cartilage (762-fold decrease, P < 0.001 and 6-fold decrease, P < 0.001, respectively, CaReS®/C210 compared to native cartilage) [Fig. 3(C, D)]. As regards the Col2/Col1 ratio, CaReS® comprised the most differentiated chondrocytes among the transplants (15-fold higher than Novocart®/C210 3D, P < 0.001). No significant differences were observed between the other scaffolds. CaReS® also exhibited the highest aggrecan/versican ratio, followed by MACI® (5.9-fold decrease, P < 0.001, compared to CaReS®), Novocart 3D (9.1-fold decrease, P < 0.001) and Hyalograft®C showing the lowest aggrecan/versican ratio (25-fold decrease, P < 0.001).

Discussion

Scaffold composition and architecture is believed to have considerable impact on the differentiation and morphology of the seeded cells. In this study we have therefore analyzed the influence of scaffold composition and structure on the expression of cartilage-specific genes in four different clinical applied graft systems. Beside the differences between the transplant types, we also evaluated their gene expression levels at the time point of transplantation in relation to native cartilage.

Comparison of native cartilage with transplants

In general, 3D conditions are believed to stimulate or preserve the differentiated stage of chondrocytes. However, despite the cultivation on three dimensional scaffolds Col2, aggrecan and MIA dramatically decreased, either as a relic of monolayer cultivation or due to inefficient differentiation on and in the scaffold. Interestingly, almost no change in Col1 expression was found, although several studies have demonstrated an increase in Col1 expression already a short time after cell isolation. In articular cartilage Col1 is expressed especially in the superficial layer. With the exception of Novocart® 3D, cartilage is collected by scratching with a sharp spoon, resulting in a disproportionately high proportion of the superficial layer and with it cells expressing Col1. In addition, the biopsies are taken from the notch, a non-weight-bearing area, in which the Col1 expression might be higher than in weight-bearing areas. For Novocart® 3D the biopsies are taken from the notch in form of cylinders and it is not specified which cells are used by the company.

Scaffold comparison

Of all analyzed scaffolds CaReS® exhibited the highest Col2, aggrecan and MIA expression, indicating a relatively high differentiation of cells compared to the other transplants. In contrast to the other scaffolds the cells are seeded in the collagen gel immediately after cell isolation, skipping the propagation step in monolayer culture, which leads to dedifferentiation. In addition, the shortest total cultivation time of all graft systems is used for the collagen gel giving the cells only a short time to propagate and dedifferentiate. Nevertheless, distinct signs of an ongoing dedifferentiation process can be observed. Beside the highly diminished Col2 and aggrecan expression compared to native cartilage, many cells appear elongated in histology. This cell shape may be supported by the specific scaffold characteristics. As suggested by
Nürnberger et al., the dense network of small collagen fibres supports a flattened cell shape and the formation of cell extensions\textsuperscript{42}.

Hyalograft\textsuperscript{C} had the highest variance in matrix gene expression. This may result from the longest and most variable cultivation time of all analyzed scaffolds, which implicates that the cells undergo variable cell doublings and passages to reach the right cell number needed for seeding at the target density. Different interactions of the cells with the scaffold’s hyaluronan fibres could also contribute to the high variance in gene expression, since a concentration dependent influence of hyaluronan on chondrocyte proliferation and proteoglycan production has been demonstrated in several studies\textsuperscript{43,44}. Another property of the scaffold is its open organisation with large space between the hyaluronan fibres. Therefore the cellular behaviour is only lightly guided by the scaffold, resulting in a high dependence on interindividual differences of the cells. Binding of the cells to hyaluronan is achieved by the CD44 receptor. A recent paper of our working group demonstrated

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Real-time PCR analysis of MIA (A) and IL-1β (B) expression in four different transplant types [MACI\textsuperscript{C} (n = 22), Hyalograft\textsuperscript{C} (n = 91), CaReS\textsuperscript{5} (n = 26) and Novocart\textsuperscript{3D} (n = 26)] and in cartilage biopsies (n = 26). Due to the overall low IL-1β expression only IL-1β positive samples were included into the analysis [MACI\textsuperscript{C} (n = 16), Hyalograft\textsuperscript{C} (n = 69), CaReS\textsuperscript{5} (n = 5), Novocart\textsuperscript{3D} (n = 24), cartilage biopsies (n = 6)]. The Col2/Col1 (C) and aggrecan/versican (D) ratios were calculated by division of Col1 and versican expression by Col2 and aggrecan expression, respectively. Gene expression was normalized to B2M. The gene expression values were plotted in a boxplot diagram (black line = median; top of grey box = 25. percentile, bottom of grey box = 75. percentile, whisker = 1.5-fold box height or minimum and maximum value respectively, dot = outlier, asterisk = extreme outlier).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Correlation between Col2 and aggrecan expression (A) and Col2 and MIA expression (B). The relative Col2 expression of all transplant and biopsy samples were plotted on the y-axis vs the relative aggrecan (A) or MIA (B) expression of all transplant and biopsy samples on the x-axis.}
\end{figure}
an increased expression level of CD44 during monolayer culture indicating a high binding capacity of the dedifferentiated cells to the hyaluronic fibres. This would explain why the cells are very equally distributed, despite the open organisation of the scaffold and subsequent difficulties for cell seeding.

In Novocart3D, the low expression of all matrix components may be due to the short cultivation time of the cells on the scaffold before shipping. In contrast to the other grafts, the cells are seeded on the scaffold only 2 days before shipping, so the cells have only little time to adapt to the new culture conditions, which might make the cells more vulnerable to the transport. This stress may lead to the lowest gene expression, except for MIA, in all analyzed scaffolds. We can therefore speculate that the cells are only temporarily metabolically inactive. However, regarding the Col2/Col1 ratio the cells exhibit the second highest differentiation, which correlates with the morphology of the chondrocytes: most of them appear spherical and only some exhibit a polygonal cell shape.

In MACI™ the cells showed a comparable high Col1 expression in the transplants. This particularity can be explained by the behaviour of the cells on that scaffold, which do somewhat invade the scaffold material, but tend to form cell-multilayer on the scaffold surface. The cells in the multilayer are elongated and fibroblast-like as an example and probably responsible for the high production of Col1 compared to the other scaffolds. On the other hand, several studies have demonstrated that high density cultures can retain the differentiation of chondrocytes probably a sign of ongoing differentiation.

In general, IL-1, a proinflammatory cytokine, is expressed in osteoarthritic cartilage and is known to induce cartilage degradation. Since this factor may also negatively affect the transplant, IL-1 expression was analyzed in the transplant samples. Monolayer culture of human chondrocytes was shown to increase IL-1 expression. We couldn’t find any significant differences in IL-1β expression between the biopsies and cartilage transplants indicating that the expression levels are restored by 3D culture conditions. This observation, however, may also result from the low number of cartilage biopsies, from which only six were positive for IL-1β. Between the scaffolds there were obvious differences in IL-1 expression, showing low levels of IL-1β in CaReS® and Novocart3D and higher levels in Chondrogide and Hyalograft®C. The higher expression in the two latter grafts could be caused by the long cultivation of cells in monolayer culture implicating a higher passage number. In addition to the high expression of IL-1β in Hyalograft®C, a huge variance in expression was noticeable between the individual samples. This variance may result from interactions with hyaluronan, as this glycosaminoglycan was demonstrated to modulate inflammation and IL-1β expression dependent on its molecular weight. Since IL-1β expression was generally low in all samples and in order to avoid false positive samples, only samples that were positive for IL-1β were included into these analyses. Specifically, IL-1β couldn’t be detected in many of CaReS® and biopsy samples. An even lower level of expression can therefore be assumed for those groups.

To our knowledge this is the first study comparing more than two different, clinically used transplant types on the molecular dependent on its molecular weight. Since IL-1β expression was generally low in all samples and in order to avoid false positive samples, only samples that were positive for IL-1β were included into these analyses. Specifically, IL-1β couldn’t be detected in many of CaReS® and biopsy samples. An even lower level of expression can therefore be assumed for those groups.

To our knowledge this is the first study comparing more than two different, clinically used transplant types on the molecular level. In contrast to histological and morphological scores, gene expression results by real-time PCR have the advantage of being person-independent. However, gene expression analysis represents the whole sample – not differing between sample regions or single cells. In addition, the number of cells is not taken into account, but could influence the results. A large difference in cell count can be assumed between the scaffolds, due to the highly different culture durations and conditions by the companies. Data about the influence of cell number or cell differentiation on the outcome of chondrocyte transplantation are still missing. As a result we do not know if it is more important to accept low cell numbers for high differentiated cells, or if a high cell number of more dedifferentiated cells is required to rebuild the ECM of the cartilage defect. For this reason our results are mostly descriptive and we cannot predict the clinical outcome on the basis of our results. Large prospective clinical studies comparing different grafts would be needed to reveal the influence of the analyzed factors on the clinical outcome after transplantation. Once clinically relevant values are defined, real-time PCR could serve as an easy way for controlling transplant criteria before implantation.

Conclusion

Our data demonstrates that the gene expression and cell differentiation differ highly between the analyzed scaffolds at the time point of transplantation. These differences seem to result from scaffold characteristics as well as from culture conditions, e.g., highly variable passage numbers, by the companies. Despite the cultivation under 3D conditions, the cell differentiation on all transplant types didn’t reach the levels of native cartilage. Further clinical trials comparing the different transplant types are needed to clarify the influence of the analyzed parameters on the clinical outcome.

Contributions

The corresponding author Christian Albrecht designed the study together with Stefan Marlovits. The acquisition of molecular data was conducted by Brigitte Tichy and Stefanie Hosiner. Electron microscopy was performed by Sylvia Nürnberger. Data analysis and interpretation was conducted by Christian Albrecht, Brigitte Tichy, Lukas Zak and Silke Aldrian. Christian Albrecht drafted the article and all the other authors revised the manuscript for important intellectual content. All authors approved the final version to be submitted.

Conflict of interest

The authors report no conflicts of interest.

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