Bone & Cartilage Regeneration
News 01|09

CONTENTS

GEISTLICH SURGERY INSIGHTS
02 Introduction

BONE & CARTILAGE REGENERATION
03 Fibrin Glue in Cartilage Regeneration
08 AMIC® Registry Update
10 Case Report Metatarsal Defect
11 Case Report OCL Talus
12 Posters and Abstracts

CONGRESSES AND EVENTS
18 Preview 2009

DISTRIBUTION
Introduction

Geistlich Surgery is pleased to present you the 9th issue of its bone and cartilage newsletter. In this issue we present developments in cartilage regeneration with a focus on autologous matrix induced chondrogenesis.

During the last congresses in Switzerland and Germany, cartilage repair was a prominent topic. We have reviewed and included selected publications and abstracts in this newsletter highlighting the advances in cartilage regeneration. Amongst the topics discussed were diagnosis and treatment options for cartilage defects in the talus and forefoot. Two of the cases presented have kindly been placed at our disposal for publication in our newsletter. Both cases show a fascinating development of the AMIC® technique in extended areas of indication. We look forward to more results of the AMIC® technique in the foot and ankle.

Controversial data exists concerning the usage of fibrin glue in cartilage and the influence on chondrocytes and bone marrow stem cells (BMSC’s). We conducted an in-vitro study at the university of Heidelberg testing fibrin glue, also in conjunction with Chondro-Gide®. The study showed that Chondro-Gide® is not only a suitable cell carrier but seems to positively influence chondrogenic differentiation of BMSC and even stimulates chondrocytes to enhance proteoglycan deposition in combination with fibrin glue.

An interesting paper by Steck et. al., also from Heidelberg, investigated the differentiation behaviour of BMSC’s in cartilage defects covered with Chondro-Gide® in an in vivo study using Göttingen mini pigs. The spontaneous BMSC differentiation was analysed during 8 weeks and the results showed that these support cartilage repair in an early phase and that the spontaneous chondrogenic differentiation of the cells in the defect also appeared to be accelerated.

Neumann et. al. that the multipotent mesenchymal progenitor cells, which are found in the subchondral cortico-spongious bone, have the capacity to undergo chondrogenic lineage development and may contribute to the formation of a cartilaginous repair tissue after microfracture treatment, which is also applicable to AMIC®.

In August 2008 we launched the new version of the AMIC® Registry, an internet database for a real world follow-up of AMIC® knee patients. A summary of the results from all centres has been included and offers a good comparison to data from MACT patients as published by Anders et. al. (2008). We encourage you to actively participate in gathering further data in the future and including this into the registry.

Last but not least, we would like to take this opportunity to offer you a platform within this newsletter to present interesting cases in bone and cartilage regeneration in the form of case reports in future copies.

The natural matrices from Geistlich Surgery have proven the efficacy in regeneration of bone and cartilage and are the natural choice of leading orthopaedic surgeons around the world.

Geistlich Surgery
January 2009
Fibrin Glue in Cartilage Regeneration

Influence of fibrin glue and partial autologous fibrin glue on chondrogenic differentiation of human bone marrow-derived stem cells and chondrocytes

Clinical Research Department—Geistlich Surgery

1. Introduction
Geistlich Pharma AG is promoting a new cartilage repair technique – AMIC® (Autologous Matrix Induced Chondrogenesis) – whereby bone marrow derived mesenchymal stem cells (BMSC) are recruited into the prepared defect area through microfracturing. The resulting, so called super clot, is stabilized by a collagen type I/III matrix (Chondro-Gide®, Geistlich Pharma AG, Switzerland) which covers the defect and is mostly fixed with fibrin glue (FG) or partial autologous fibrin glue (PAF). PAF is prepared through the substitution of parts of the thrombin with autologous patient serum to increase the TGF-β content, known to play a crucial role in chondrogenesis.

Since there is controversial data/literature about the behaviour of BMSCs in fibrin sealants Geistlich Surgery organized an in vitro study elucidating whether commercially available fibrin glue (Tissucol, Baxter, Germany) and its partial autologous version support the proliferation and chondrogenic differentiation of human bone marrow-derived stem cells when combined with a collagen type I/III matrix Chondro-Gide®.

Human articular chondrocytes, as used in cell based cartilage repair techniques like Autologous Chondrocytes Implantation (ACI), were chosen as a reference.

2. Materials and Methods
The study was conducted at the University Clinic Heidelberg, department experimental orthopaedics, headed by Prof. Dr. Wiltrud Richter. It was approved by the local ethics committee and Informed Consent was obtained from all individuals donating either bone marrow or cartilage samples.

2.1 Isolation and Expansion of Chondrocytes and BMSC
Human articular chondrocytes were obtained from five patients (age 65 ± 10 years) undergoing total knee replacement surgery. Cartilage was harvested from regions with no evident degeneration. Chondrocytes were plated at a cell density of 6 × 10³ cells/cm² and expanded for 2 passages.

Bone marrow-derived stem cells (BMSC) were isolated from fresh bone marrow samples obtained from three patients (age 55 ± 14 years) undergoing total hip replacement. Cells were fractionated by density gradient centrifugation and seeded in culture flasks. For expansion BMSC were plated at 6 × 10⁴ cells/cm² in monolayer culture and were expanded for 3 passages.

2.2 Set-up and Chondrogenic Culture
The following test groups were chosen:

| Group 1 - Pellet (P): | P, P + FG, P + PAF |
| Group 2 - Matrix (M): | M, M + FG, M + PAF |

After expansion in monolayer, chondrocytes or BMSCs were harvested using trypsin/EDTA. Carrier-free pellets consisting of 0.5 × 10⁶ cells were formed by centrifugation (P, P+FG and P+PAF) for a first group.

In a second group, 4 mm round disks from collagen type I/III matrix Chondro-Gide® were charged with 10×10⁶ cell suspension containing 0.5 × 10⁶ cells and incubated for 1 hour at 37°C before the FG or PAF was applied (M+FG, M+PAF). BMSCs were also tested on Chondro-Gide® without fibrin glue (M).

Pellets and matrices were cultured for 4 weeks (gene expression analysis) or 6 weeks (histology and analysis of proteoglycan content) with 500 μl chondrogenic medium per pellet or matrix. Medium was changed three times a week.
After 6 weeks pellets and matrix constructs were evaluated using Histology (collagen type II staining, semiquantitative analysis through two independent observers based on a score), relative proteoglycan (PG) content determined by alcian blue quantification and RNA isolation and quantitative real time PCR (gene expression).

3. Results
The results presented here are shortened and only Histology and PG content are discussed. A full overview is given in a publication submitted but currently under review.

3.1 Chondrocytes
Pellets and membrane constructs prepared with chondrocytes showed a strong collagen type II staining for all donors (D). Wilcoxon test was used for statistical analysis.

The scores obtained for the pellets alone were significantly higher than the scores obtained for all other pellets and matrix constructs (table 1). The scores for the M+FG were significantly lower than the scores for FG pellets and the scores for the M+PAF were significantly lower than the scores for the PAF pellets. Matrix constructs prepared with PAF reached significantly higher scores than matrix constructs prepared with FG indicating that PAF stimulated collagen type II deposition and in part compensated for the reduction of collagen type II staining intensity in the matrix constructs.

Quantitative analysis of proteoglycans deposited within pellets and matrix constructs revealed a significantly lower proteoglycan content for carrier-free pellets compared to all other pellets and constructs. A similar PG content was found in FG pellets, PAF pellets and M+FG or PAF derived from all donors demonstrating that both FG and PAF alone and in combination with Chondro-Gide® supported matrix deposition (figure 1).

Table 1: Collagen type II staining scores of pellets, FG pellets, PAF pellets, matrix with FG and M with PAF prepared with chondrocytes derived from 5 donors.

<table>
<thead>
<tr>
<th></th>
<th>Pellet</th>
<th>Pellet FG</th>
<th>Pellet PAF</th>
<th>Matrix FG</th>
<th>Matrix PAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>D 1</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>1.00</td>
<td>2.00</td>
</tr>
<tr>
<td>D 2</td>
<td>3.00</td>
<td>2.75</td>
<td>2.25</td>
<td>1.50</td>
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</tr>
<tr>
<td>D 3</td>
<td>3.00</td>
<td>3.00</td>
<td>2.50</td>
<td>2.00</td>
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</tr>
<tr>
<td>D 4</td>
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<td>2.75</td>
<td>2.00</td>
<td>3.00</td>
</tr>
<tr>
<td>D 5</td>
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<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Mean</td>
<td>3.00</td>
<td>2.78 (^a)</td>
<td>2.72 (^a)</td>
<td>1.625</td>
<td>2.40 (^b)</td>
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<td>SD</td>
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<td>0.48</td>
<td>0.51</td>
<td>0.71</td>
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</tbody>
</table>

\(^a\) significantly different compared to P, \(^b\) significantly different to M+FG (p < 0.05)

Chondrocytes: Relative proteoglycan content

Figure 1: Relative proteoglycan content determined by alcian blue quantification of pellets and matrix constructs prepared with chondrocytes derived from 5 donors at 6 weeks of culture under chondrogenic conditions. Mean and standard deviation of pellets and matrices of all donors are given.
3.2 Mesenchymal stem cells from bone marrow (BMSC)

The results in the BMSC group varied between the different donors.

Statistical analysis by Wilcoxon test in which carrier-free pellets, FG pellets and PAF pellets were compared revealed no significant differences between these groups. Similarly no statistically significant differences were found between Chondro-Gide® matrix alone, matrix constructs with FG and with PAF (table 2). Generally values in the matrix group were higher than for the pellet group.

<table>
<thead>
<tr>
<th></th>
<th>Pellet</th>
<th>Pellet FG</th>
<th>Pellet PAF</th>
<th>Matrix</th>
<th>Matrix FG</th>
<th>Matrix PAF</th>
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<tr>
<td>D 2</td>
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<td>0.75</td>
<td>0.50</td>
<td>2.00</td>
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<td>1.25</td>
</tr>
<tr>
<td>D 3</td>
<td>1.50</td>
<td>1.50</td>
<td>1.00</td>
<td>2.00</td>
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<tr>
<td>Mean</td>
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<td>0.85</td>
<td>0.00</td>
<td>0.47</td>
<td>0.50</td>
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</tbody>
</table>

Table 2: Collagen type II staining scores of pellets, FG pellets, PAF pellets, matrix, matrices with FG and matrices with PAF prepared with BMSC derived from 3 donors.

Exemplary below, histology of bone marrow derived stem cells from donor 3.

In sum, seeding of BMSC on a collagen I/III matrix seemed to strongly favour collagen type II deposition. This was especially evident when differentiation capacity of the donor cells was low. The additional application of FG or PAF had no strong effect on differentiation of BMSC with a good differentiation capacity while chondrogenesis of BMSC with a lower differentiation capacity was slightly negatively affected by PAF.
Quantitative analysis of proteoglycans deposited within pellets and matrix constructs revealed significantly higher relative proteoglycan contents in FG pellets, PAF pellets and matrix constructs with or without FG/PAF compared to carrier-free pellets.

In comparison to FG pellets only the matrix constructs without FG/PAF and M+FG, but not M+PAF displayed a significantly higher proteoglycan content. These findings indicate that both FG and the collagen I/III matrix Chondro-Gide® enhanced proteoglycan deposition. These additive stimulative effects were not observed when autologous serum was applied to the matrices in addition to FG since proteoglycan deposition in M+PAF was not significantly higher than proteoglycan deposition in FG pellets. Nevertheless M+PAF displayed a significantly higher PG deposition compared to PAF pellets confirming the positive effect of the matrix on proteoglycan deposition.

**4. Conclusion**

**4.1 Chondrocytes**

The results obtained from this study demonstrate that fibrin glue and partial autologous fibrin glue stimulate chondrocytes to deposit more proteoglycans in the newly formed extracellular matrix independent of whether the cells were grown in pellet culture or on the collagen type I/III matrix Chondro-Gide®.

While gene expression including COL2A1 and aggregcan mRNA levels were little affected by FG and PAF, a small but significant reduction of collagen type II deposition appeared for both fibrin glues in semi-quantitative histology, again independent of whether the matrix was chosen as a carrier or not. When combined with the Chondro-Gide®, PAF however seemed to be superior to FG. This suggests that collagen type II production rates were unrelated to the use of FG and PAF while a possible difference in the deposition of collagen type II protein may occur *in vitro* in fibrin glue which could explain the slightly inferior results obtained in histology.

In sum, better arguments for than against the application of commercially available fibrin glue (Tissucol, Baxter, Germany) in cell based cartilage repair strategies like ACI can be found, with slightly better results obtained for PAF versus FG regarding proteoglycan and collagen type II deposition in the collagen I/III matrix Chondro-Gide®.

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**Figure 3:** Relative PG content determined by alcian blue quantification of pellets and matrix constructs prepared with BMSC derived from 3 donors at 6 weeks of culture under chondrogenic conditions. Mean and standard deviation of pellets and matrix of all donors are given.

a) significantly different to pellet, b) significantly different to FG pellet, c) significantly different to PAF pellet (p < 0.05).
4.2 Mesenchymal stem cells from bone marrow (BMSC)

In the experiments on *in vitro* chondrogenesis of BMSC all three additions – FG, PAF and Chondro-Gide® - improved proteoglycan deposition compared to pellet alone. Even better results were seen, when FG or PAF were combined with the Chondro-Gide® matrix.

Gene expression provided a trend to higher COL2A1 mRNA levels in Chondro-Gide® with some reduction observed when FG and PAF were applied additionally. Collagen protein deposition by BMSC showed the same trend, which did, however, not reach statistical significance.

In contrast to chondrocytes, no argument to recommend PAF instead of FG can be obtained from this *in vitro* study for BMSC in combination with Chondro-Gide®.

In summary, Chondro-Gide® showed a convincing support of bone marrow derived stem cells in *in vitro* chondrogenesis and may be favourable for chondrogenic differentiation of BMSC also when combined with FG or PAF.

Even though the study was designed to mainly test the influence of fibrin glue on BMSC it could also be proven, that cells when combined with a carrier, do better. Chondro-Gide® provides not only a suitable cell carrier but seems to positively influence chondrogenic differentiation of BMSC and even stimulate chondrocytes to enhance proteoglycan deposition in combination with fibrin glue.

In conclusion, the use of the commercially available fibrin glue Tissucol for the fixation of the collagen I/III matrix Chondro-Gide® in cartilage repair with AMIC® and ACI can be regarded as safe and efficient.
AMIC® Registry Update

In August we launched the new version of the AMIC® Registry. Apart from the sophisticated web interface, the clear structure, easy patient management and detailed reporting, the AMIC® Registry now offers you a wide variety of functions.

The functions at a glance:
- Internet database for AMIC® in the knee
- Patient evaluation based on the Lysholm Knee Score, VAS Pain Scale and MRT analysis
- Simple and rapid data collection
- Standardised structure of patient data with streamlined, intuitive web interface
- Clinical progress of individual patients as well as all patients from your centre
- Improved report compilation and online analysis for single patients and patient population
- Data export to Excel for further analysis
- Direct copying of diagrams into your presentations
- Patient management with autonomous activation of new patient ID
- Network to other AMIC® users via an internal mail system
- Opportunity to upload images (e.g. MRI, OR-images)
- Document management with literature, brochures and scores available for download
- Direct contact to Geistlich Surgery for support
- Newsletter with an anonymous analysis of all patients from all users
- Encrypted, anonymous data entry using a secure communication protocol

The web-based interface gives you access to all features and all data from any internet-enabled computer. The AMIC® Registry can be used in English or German. Other languages will be supported in future.

All features can be accessed from the convenient and hierarchical tree view menu. Using this menu, all entries are easily entered in a structured fashion. Apart from the initial visit and yearly follow-up results, interim visits can be documented. Images, for example MRI images, can easily be uploaded to complement the patient diagnosis.

For the patient evaluation the Lysholm Knee Score, VAS Pain Score and MRI analysis are available. The chronological workflow and pull down menus enables rapid entry of your data.

For every patient, or your patient population, the results can easily be displayed through the click of a button as an online graph or as a printed report.

If you would like to actively use and profit from the AMIC® Registry or if you have any queries regarding the AMIC® Registry, please feel free to contact Geistlich Surgery.
AMIC® Registry Newsletter

The recent AMIC® Registry newsletter included 2 to 5 year results after AMIC® in the knee. The following is an excerpt of the newsletter.

Summary

179 patients (67 female, 112 male, age 14–64 years, mean 36.8 years) with focal cartilage defects of the knee and were included in the recent analysis.

The mean defect size was 3.8 ± 2.2 cm², 99% with Grade III (n=43) or IV (n=90) according to the Outerbridge Classification. Most of the defects were allocated to the medial femoral condyle, followed by patella and lateral femoral condyle (Figure 1).

52% of all patients had at least one previous surgery on the affected knee. Lavage and debridement constituted for 34%, diagnostic arthroscopy for 30.9% and microfracture 16% of these interventions.

Excellent 2-year results (n=57) showed a mean improvement of knee function, assessed by the Lysholm Score, from 50 to 85 points and pain reduction from 6.7 to 2.0 (VAS) (Figure 2).

After 3 Years (n=28) the mean Lysholm Score improved from 56.6 to 87.0 points. Pain on a visual analog scale (VAS) diminished from 6.2 to 2.3 (Figure 3).

A total of 75% of the patients treated showed excellent to good results 3-years post-op. 10.7% obtained a satisfactory result and 14.3% an unsatisfactory result. No intra-operative data or reasons for the latter were entered into the registry.

A 4-year analysis (n=13) showed that the Lysholm Score improved from 51 to 78 points. The VAS decreased from 7.4 to 2.5 points.

The results after two years demonstrate a very good clinical outcome for the patient population with stable continuation of the good results after three years.

Please contact us if you would like a copy of the AMIC® Registry newsletter.
Reconstruction of a Cartilage Defect on the Head of the First Metatarsal

Clinical Case by Prof. Dr. med. Markus Walther, Orthopädische Klinik München-Harlaching, Germany

**Patient History, Clinical Diagnosis**
Condition after an hallux OP 2 years prior, with increasing pain in the basal joint of the big toe. Load and movement-dependant pain was indicated as 5 out of 10 on the VAS. Sport was not possible. Suspected irritation by the metal and/or cyst formation following a radiological examination. The CT showed a subchondral cyst approx. 7 mm in diameter in the head of the first metatarsal bone with a collapse of the subchondral cortical bone (Fig. 1). External suggestion to the patient was metal removal with arthrodesis of the first metatarso-phalangeal (MTP) joint.

**Operation**
A 8mm cyst was revealed intra-operatively in the area around the tip of the interterferential screw in the metatarsal head. During movement of the toe, an impingement of mobile cartilage (a 5x7 mm cartilage segment) was observed (Fig. 2).

The operation performed consisted of hardware removal and debridement of loose cartilage. The cyst was filled with cancellous bone from the iliac crest. The cartilage defect in the metatarsal head was covered with Chondro-Gide® according to the AMIC® technique (Fig. 3).

**Results, Evaluation, Progress**
Subsequent treatment consisted of wearing a post-op shoe for 8 weeks. At the same time, careful mobilization of the first MTP joint was started with traction from the 3rd day. Compared to pre-operatively, the pain level was reduced 6 months postoperatively from 5 to 0 in the VAS under everyday loads and the dorsal/plantar mobility in the large toe basal joint was 40-0-30°. It was recommended that the patient abstains from sports for 6 months and then starts with sports such as cycling, inline skating, walking and swimming. Sports which exert a high load on the forefoot (e.g. step aerobics, squash, tennis, jogging) were only permitted after 1 year.

**Discussion and Conclusions**
Chondro-Gide® offers a good possibility to cover local cartilage defects in critical places, e.g. the first MTP joint. Whether the use of the matrix is superior to cancellous bone grafting alone will only emerge in the long-term. The goal of avoiding an arthrodesis of the large toe basal joint was achieved. So far, there are only a few reports about the reconstruction of small joints, for example using rib cartilage.
Reconstruction of a Cartilage Defect of the Talus

Clinical Case by PD Dr. med. Dr. phil. Victor Valderrabano, MD PhD, Associated Professor, Orthopaedic Department, University Hospital Basel, Switzerland
Correspondence: vvalderrabano@uhbs.ch

Osteochondral lesions (OCL) of the talus is an often encountered problem in young, sportively active patients with a large impact. Apart from an accurate diagnosis of the osteochondral lesion, the OCL-promoting biomechanical factors need to be attentively analysed: ligament instability, posterior foot alignment, vascularisation. The AMIC® technique offers a new therapy option for OCL. By covering the debrided and microfractured defect with the collagen matrix Chondro-Gide®, an improved milieu for the differentiation of the MSC is created and a protective environment is formed at the site of the lesion.

Patient History, Clinical Diagnosis
A 36 year old patient presented herself with a chronic medial instability of the hindfoot a pes planovalgus et abductus deformity (A). Conventional radiography (B) revealed an osteochondral lesion of the medial shoulder of the talus. This could be confirmed using MRI (C) and SPECT-CT (D).

Operation
After debridement, microfracture and filling of the osseous defect with cancellous bone, the Chondro-Gide® matrix was attached using fibrin glue (E, F, G). The pathobiomechanical predisposed factors (ankle joint instability und pes planovalgus et abductus) were corrected by a medial reconstruction of the ligaments and lateral calcaneal lengthening osteotomy (H, I).

The post operative treatment included 15kg partial weight bearing for 8 weeks whilst wearing a stable orthosis and CPM (continuous passive motion) as part of a standardised physiotherapy rehabilitation program.

Discussion and Conclusion
The evidence level in literature is low regarding OCL therapy of the talus. Our patient collective show promising results after treatment using the AMIC® concept in conjunction with a repair of pathobiomechanical factors. The majority of the patients reported a substantial decline of symptoms and a return to sports. The AMIC® technique represents a promising treatment option for osteochondral lesions of the talus. Future studies of high evidence level for the evaluation of this and other talus OCL treatment options are necessary.
Surgical suturing of articular cartilage induces osteoarthritis-like changes

Hunziker EB, Stähli A.

Introduction
In clinical tissue-engineering-based approaches to articular cartilage repair, various types of flap are frequently used to retain an implanted construct within the defect, and they are usually affixed by suturing. We hypothesize that the suturing of articular cartilage is associated with a loss of chondrocytes from, and osteoarthritis-like changes within, the perisutural area.

Materials and methods
We established a large, partial-thickness defect model in the femoral groove of adult goats. The defects were filled with bovine fibrinogen to support a devitalized flap of autologous synovial tissue, which was sutured to the surrounding articular cartilage with single, interrupted stitches. The perisutural and control regions were analyzed histologically, histochemically and histomorphometrically shortly after surgery and 3 weeks later.

Results
Compared to control regions, chondrocytes were lost from the perisutural area even during the first few hours of surgery. During the ensuing 3 weeks, the numerical density of cells in the perisutural area decreased significantly. The cell losses were associated with a loss of proteoglycans from the extracellular matrix. Shortly after surgery, fissures were observed within the walls of the suture channels. By the third week, their surface density had increased significantly and they were filled with avascular mesenchymal tissue.

Conclusions
The suturing of articular cartilage induces severe local damage, which is progressive and reminiscent of that associated with the early stages of osteoarthritis. This damage could be most readily circumvented by adopting an alternative mode of flap affixation, such as gluing with a biological adhesive.
Repair of Large Chondral Defects of the Knee With Autologous Chondrocyte Implantation in Patients 45 Years or Older

Rosenberger RE, Gomoll AH, Bryant T, Minas T.

Background
Autologous chondrocyte implantation (ACI) has become an accepted option for the treatment of chondral defects in carefully selected patients. Current recommendations limit this procedure to younger patients, as insufficient data are available to conclusively evaluate outcomes in patients older than 45 years.

Hypothesis
Cartilage repair with ACI in patients older than 45 years results in substantially different outcomes than those previously reported for younger age groups.

Study Design
Case series; Level of evidence, 4.

Methods
This prospective cohort study reviewed patients ≥45 years of age at the time of treatment with ACI. The clinical evaluation included a patient satisfaction questionnaire and four validated rating scales: Short Form-36, Modified Cincinnati Rating Scale, WOMAC (Western Ontario and McMaster Universities) Osteoarthritis Index, and the Knee Society Score.

Results
A total of 56 patients ≥45 years of age were treated with ACI. The average patient age at index surgery was 48.6 years (range, 45-60 years). The minimum follow-up was 2 years (range, 2-11 years; mean, 4.7 years). The cohort included 36 men and 20 women. The mean transplant size was 4.7 cm² per defect (range, 1-15.0 cm²) and 9.8 cm² per knee (range, 2.5-31.6 cm²). Twenty-eight patients (50%) underwent concomitant osteotomies to address malalignment. There were 8 failures (14%): 6 of 15 (40%) in patients receiving workers' compensation (WC) and 2 of 41 (4.9%) in non-WC patients. Additional arthroscopic surgical procedures were required in 24 patients (43%) for periosteal-related problems and adhesions; 88% of these patients experienced lasting improvement. At their latest available follow-up, 72% of patients rated themselves as good or excellent, 78% felt improved, and 81% would again choose ACI as a treatment option.

Conclusion
Our results showed a failure rate of ACI in older patients that is comparable with rates reported in younger patient groups. The procedure is associated with a substantial rate of reoperations, mostly for the arthroscopic treatment of graft hypertrophy, similar to that in younger patients.
Background
Although autologous chondrocyte implantation (ACI) is a well-established therapy for the treatment of isolated cartilage defects of the knee joint, little is known about typical complications and their treatment after ACI.

Hypothesis
Unsatisfactory outcome after ACI is associated with technique-related typical complications.

Study Design
Case series; Level of evidence, 4.

Methods
A total of 309 consecutive patients with 349 ACI procedures of the knee joint were analyzed. Three different ACI techniques were used: periosteum-covered ACI in 52 cases (14.9%), Chondro-Gide® (Geistlich Biomaterials, Wolhusen, Switzerland) membrane-covered ACI in 215 cases (61.6%), and a 3-dimensional matrix-associated ACI (BioSeed-C, Biotissue Technologies, Freiburg, Germany) in 82 cases (23.5%). In 52 patients, revision surgery was performed for persistent clinical problems. These patients were analyzed for defect size and location, technique of ACI, and intraoperative findings during revision surgery. The mean time of follow-up for patients after ACI was 4.5 years (standard deviation, ±1.5).

Results
Four typical major complications were identified: hypertrophy of the transplant, disturbed fusion of the regenerative cartilage and the healthy surrounding cartilage, insufficient regenerative cartilage, and delamination. These diagnoses covered a total of 88.5% of the patients who underwent revision surgery. The overall complication rate was highest in the group of patients treated with periosteum-covered ACI (P = .008). The incidence of symptomatic hypertrophy was 5.2% for all techniques and defect locations; the highest incidence was in patients treated with periosteum-covered ACI (15.4%) (P = .001). The incidence of disturbed fusion was highest in the Chondro-Gide®-covered ACI (3.7%) and the matrix-associated ACI group (4.8%). Concerning the incidence of complications by defect location, there was a tendency for increased complications in patellar defects (P = .095).

Within the patellar defects group, no correlation was found for the occurrence of delamination, insufficient regeneration, and disturbed fusion. As a statistical trend, an increased rate of hypertrophy was found for patellar defects (P = .091).

Conclusion
A major proportion of complications after ACI can be summarized by 4 major diagnoses (symptomatic hypertrophy, disturbed fusion, delamination, and graft failure). Among those, the overall complication rate and incidence of hypertrophy of the transplant were higher for periosteum-covered ACI. Furthermore, an increased rate of symptomatic hypertrophy was found for patellar defects. Therapeutic concepts need to be developed to treat these typical complications of ACI.
Objective
Repair of localized cartilage defects in the knee.

Indications
Localized partial or full-thickness cartilage defects in the knee or osteochondral lesions (osteochondritis dissecans [OD]).

Contraindications
Generalized cartilage defects, osteoarthritis, bacterial and rheumatoid arthritis, uncorrected axis deformities, ligament instability, patella instability, meniscectomy.

Surgical Technique

Postoperative Management
Early functional rehabilitation with knee orthosis. Partial weight bearing (20 kg) for 6 weeks.

Results
50 patients (24 female, 26 male, age 14–44 years, mean 30.3 years) with 58 focal cartilage defects (III–IV°) of the knee in the medial (n = 32) or lateral condyle (n = 5), patella (n = 14) and/or trochlea (n = 7) underwent matrix-associated autologous chondrocyte implantation (MACI®).

The mean follow-up was 24 months (21–29 months). The mean defect size was 4.1 cm² (1.6–6.1 cm²). The Lysholm Score improved from 57.3 to 87.4 points, the DGKKT (German Society of Autologous Cartilage and Bone Cell Transplantation) Score from 55.3 to 85.5 points. Pain on a visual analog scale (VAS) diminished from 5.5 to 2.1, while subjective function enhanced from 4.5 to 7.6. All scores were significant (p < 0.01; t-test).

In eleven patients (twelve defects), a second-look arthroscopy revealed a mostly fibrocartilaginous regenerative tissue in 41.7% (5/12) and a mixed fibrous/hyaline regenerative tissue in 33.4% (4/12). 54% (27/50) of the patients estimated their result as excellent, 28% (14/50) as good, 16% as fair, and 2% (1/50) as poor.

Discussion
The article delivers a good overview of the results and operating technique. The reported mean defect size of 4.1cm² lies in a range ideally suited for the AMIC® technique and is comparable to the results from the AMIC® Registry patients (3.8cm²). Knutsen et al. reported a mean defect size of 5.1cm² when comparing MF to ACI.

The result of the knee function as evaluated by the Lysholm Score two years post-operatively is comparable to our AMIC® Registry patient group whereas our mean pre-op Lysholm score is only 50. Pain reduction is comparable.

The second-look arthroscopy results are interesting in that 75.1% of the biopsies showed mainly fibrous cartilage repair tissue or a mixture of fibrous/hyaline regenerative tissue.

Comparing the results of this study to the patient group recorded in the AMIC® Registry, the differences in outcome are minimal at two years. The advantage of the AMIC® procedure is clearly that it is a cost-efficient, single-step operation. Long term evaluation and comparative studies will be needed to further evaluate the outcome of the MACT and AMIC® respectively. Results of a randomised study comparing different techniques would be desirable.

Spontaneous Differentiation of Autologous Mesenchymal Stem Cells in a Cartilage Defect in Göttingen minipigs*

Steck E, Lorenz H, Gotterbarm T, Jung M, Richter W
EF10-32, Free paper, Deutscher Kongress für Orthopädie und Unfallchirurgie 2008, Berlin/Germany

Hypothesis
MSC’s offer an attractive cell source for the treatment of focal cartilage defects. However, their chondrogenic differentiation in vitro also leads to a hypertrophic differentiation of these cells. This could present a risk for the phenotypic stability of the regenerative tissue, if MSC’s differentiate in the same manner in vivo. Aim of the study was to evaluate if the orthotope surrounding in the knee joint can influence the differentiation of transplanted or immigrating MSC’s toward chondrocyte and can inhibit the expression of hypertrophic markers in the repair tissue. During 8 weeks the spontaneous MSC differentiation in a cartilage defect model was there analysed on a histological and molecular biological level.

Method
MSC’s were isolated from the bone marrow of Göttingen minipigs, expanded and transplanted into “full thickness” cartilage defects. All animals were operated on both hind limbs to induce a cartilage defect which was covered using a collagen I/III matrix. In one group the autologous MSC’s were transplanted under the matrix and the second group did not receive a cell transplantation. After 1 (n=4), 3 (n=4) and 8 (n=6) weeks the repair tissue was analysed histologically and using molecular biological assay of for the expression of the cartilage markers Col2A1 and AGC and the hypertrophic markers Col10A1 and MMP13.

Results
Defect filling and integration into the surrounding tissue was incomplete after 1 and 3 weeks and none of the groups showed Safranin-O stained regeneration tissue, congruent with undetectable AGC on the level of gene expression. An incipient collagen type II staining could only be shown in the presence of transplanted MSC’s. After 8 weeks most of the regenerative tissue showed good integration in both groups. Safranin-O and collagen type II positive repair tissue could be detected in 4 of the 6 defect fillings with MSC and in 2 out of 6 without MSC transplantation accompanied by a significant increase in AGC and Col2A1 expression levels. The induction of Col10A1 or MMP13 was comparable in both groups after 8 weeks, whereas collagen type X could only be confirmed immunohistochemically in the deep area adjacent to the subchondral bone.

Conclusion
Our data substantiates that the transplanted autologous MSC’s support the cartilage repair in the early phase. A spontaneous chondrogenic differentiation of the cells in the defect appears to be accelerated through this. The expression of hypertrophic markers in the process presents a general phenomenon which in vivo is confined to the deep cartilage zones. If the hypertrophic area eventually disappears through continued remodelling of the repair tissue and whether the transplanted MSC’s are directly involved in the development or if they merely affect naturally migrating cells via secreted molecules, needs to be answered in further studies.

* Translation of the German original.
Chondrogenic Differentiation Capacity of Human Mesenchymal Progenitor Cells Derived from Subchondral Cortico-Spongious Bone

Neumann K, Dehne T, Endres M, Erggelet C, Kaps C, Ringe J, Sittinger M.

Abstract
Microfracture is frequently used to repair articular cartilage defects and allows mesenchymal progenitors to migrate from subchondral bone into the defect and form cartilaginous repair tissue. The aim of our study was to analyze the cell surface antigen pattern and the differentiation capacity of cells derived from human subchondral bone.

Human progenitor cells were derived from subchondral cortico-spongious bone and grown in the presence of human serum. Stem cell-related cell surface antigens were analyzed by flowcytometry. Corticospongious progenitor (CSP) cells showed presence of CD73, CD90, CD105, and STRO-1. Multilineage differentiation potential of CSP cells was documented by histological staining and by gene expression analysis of osteogenic, adipogenic, and chondrogenic marker genes. CSP cells formed a mineralized matrix as demonstrated by von Kossa staining and showed induction of osteocalcin, independent of osteogenic stimulation.

During adipogenic differentiation, the adipogenic marker genes fatty acid binding protein 4 and peroxisome proliferative activated receptor γ were induced. Immunohistochemical staining of cartilage-specific type II collagen and induction of the chondrocytic marker genes cartilage oligomeric matrix protein, aggrecan, and types II and IX collagen confirmed TGF-β3-mediated chondrogenic lineage development.

Conclusion
Multipotent mesenchymal progenitor cells reside in a variety of mesenchymal tissues, including the subchondral cortico-spongious bone that is accessed by microfracture. These cells, as shown here, are stem cell-like mesenchymal progenitors, have the capacity to undergo chondrogenic lineage development, may be guided into the defect area by synovial fluid or human serum, and may contribute to the formation of a cartilaginous repair tissue after microfracture treatment.

CSP cells from subchondral bone, as known from microfracture, are multipotent stem cell-like mesenchymal progenitors with a high chondrogenic differentiation potential.
Congress Preview 2009

Geistlich Surgery will be present at a number of events in the coming year. We look forward to an exciting calendar with symposia and congresses.

Events for 2009

<table>
<thead>
<tr>
<th>Congress</th>
<th>Date</th>
<th>Venue</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHH Symposium</td>
<td>13 - 14 March</td>
<td>Hannover, Germany</td>
</tr>
<tr>
<td>BASK Annual Meeting</td>
<td>02 - 03 April</td>
<td>Edinburgh, UK</td>
</tr>
<tr>
<td>Deutsche Gesellschaft für Chirurgie</td>
<td>28 April - 1 May</td>
<td>Munich, Germany</td>
</tr>
<tr>
<td>8th World Congress ICRS</td>
<td>24 - 26 May</td>
<td>Miami, USA</td>
</tr>
<tr>
<td>10th EFORT Congress</td>
<td>3 - 6 June</td>
<td>Vienna, Austria</td>
</tr>
<tr>
<td>SGO Kongress</td>
<td>24 - 26 June</td>
<td>Geneva, Switzerland</td>
</tr>
<tr>
<td>BOA—British Orthopaedic Association</td>
<td>15 - 18 September</td>
<td>Manchester, UK</td>
</tr>
<tr>
<td>26th AGA Kongress</td>
<td>17 - 19 September</td>
<td>Leipzig, Germany</td>
</tr>
<tr>
<td>DGU/DGOOC</td>
<td>21 - 24 October</td>
<td>Berlin, Germany</td>
</tr>
<tr>
<td>Eurospine</td>
<td>21 - 24 October</td>
<td>Warsaw, Poland</td>
</tr>
<tr>
<td>SIOT</td>
<td>07 - 11 November</td>
<td>Milan, Italy</td>
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</tbody>
</table>

Further Information

Please consult our web page for further information and invitations to the lunch symposia.

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