Single-stage cartilage repair in the knee with microfracture covered with a resorbable polymer-based matrix and autologous bone marrow concentrate

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Abstract

Background: Different single-stage surgical approaches are currently under evaluation to repair focal cartilage lesions. This study aims to analyze the clinical and histological results after treatment of focal condylar articular lesions of the knee with microfracture and subsequent covering with a resorbable polylactic acid/hyaluronic acid (PGA-HA) matrix augmented with autologous bone marrow concentrate (BMC).

Methods: Nine patients with focal lesions of the condylar articular cartilage were consecutively treated with arthroscopic PGA-HA-covered microfracture and bone marrow concentrate (PGA-HA-CMBMC). Patients were retrospectively assessed using standardized assessment tools and magnetic resonance imaging (MRI). Five patients consented to undergo second look arthroscopy and 2 consented biopsy harvest.

Results: All the patients but one showed improvement in clinical scoring from the pre-operative situation to the latest follow-up (average 22±2 months). The mean IKDC subjective score, Lysholm score, VAS and the median Tegner score significantly increased from baseline to the latest follow-up. Cartilage macroscopic assessment at 12 months revealed that one repair appeared normal, three almost normal and one appeared abnormal. Histological analysis proved hyaline-like cartilage repair tissue formation in one case. MRI at 8 to 12 months follow-up showed complete defect filling.

Conclusions: The first clinical experience with single-stage treatment of focal cartilage defects of the knee with microfracture and covering with the PGA-HA matrix augmented with autologous BMC (PGA-HA-CMBMC) suggests that it is safe, it improves knee function and has the potential to regenerate hyaline-like cartilage.

Level of evidence: IV, case series.

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1. Introduction

Focal cartilage defects occur frequently and are a common cause of knee symptoms and disability, and may progress to severe osteoarthritis (OA) [1,2]. Therefore, an ideal cartilage repair procedure should recreate hyaline-like cartilage, ultimately prevent OA [3] and restore the articular surface. Different surgical options are now available to treat cartilage defects, which have to be chosen mainly according to defect size, patient functional needs and expected cost-effectiveness. Among others, the microfracture (MFX) treatment is a commonly used and cost effective first-line treatment option for focal cartilage defects [4,5]. In addition, autologous chondrocyte implantation (ACI) and matrix and/or scaffold-assisted ACI [6–10] are regarded as second-line treatment for small and a first line option for defects larger than two to four centimetres squared [11].

The limits of the MFX treatment are with respect to lesion size and to long term functional improvements [3,12]. However, high costs and the need for two interventions in ACI and ACI-related procedures [13] have prompted the search for new and improved single-stage cartilage repair methods. Autologous matrix-induced chondrogenesis (AMIC) has emerged as a new technique utilizing a porcine collagenic scaffold combined with fibrin glue, autologous serum and microfractures [14,15]. Newer procedures favour synthetic polymer scaffolds like PGA–HA scaffolds for covering of microfractured defects have shown the potential to regenerate hyaline-like cartilage [10,16–18]. All these techniques have in common that the microfractures should allow for the in-growth of mesenchymal progenitor cells from the subchondral bone into the scaffolds, enrich the cells within the defect and guide them toward cartilaginous tissue formation [19].

Since the number of stem or progenitor cells may be reduced with age [20] and subchondral progenitors may show a low potential to form hyaline-like repair tissue in early osteoarthritis [21], the enrichment of the defect with autologous BMC or bone marrow-derived cells seems to be attractive. In particular BMC from the iliac crest may be of interest, since twice the percentage of cells show mesenchymal stem cell markers compared to cells harvested from blood during the microfracture procedure [22]. Recently, it has been...
shown that intra-articular application of iliac crest BMC and marrow aspirate in hyaluronan improved the outcome of the microfracture treatment in full thickness cartilage defect, in the horse model [23] and in the goat model [24]. These findings, for instance, have led to modification of the original single-stage technique involving the addition of BMC to treat talar osteochondral lesions [25].

In the present pilot study, chondral cartilage lesions have been treated with MFX and defects were covered with PGA–HA scaffolds immersed with autologous BMC from the iliac crest. The aim of this study is to analyze the clinical and histological outcome of PGA–HA-covered microfractures and bone marrow concentrate (PGA–HA-CMBMC) [26].

Fig. 1. Arthroscopic technique. a The cartilage defect is identified; b debrided; c and measured. d Microfracture is performed with the appropriate awl. e The water flow is stopped; f and the mixture of fibrin glue and BMC is deposited on the bed of the defect. g The PGA–HA matrix immersed with BMC is set in place with a probe; h and covered with the rest of the fibrin glue–BMC mixture injected through a long needle. i Final appearance of the repaired defect.
2. Materials and Methods

2.1. Study design

From April to October 2010, nine consecutive patients with symptomatic chondral lesions of the knee underwent arthroscopic MFX and implantation of the PGA–HA matrix (Chondrotissue®, BioTissue AG, Zurich, Switzerland) seeded with autologous BMC from the iliac crest (PGA–HA-CMBMC). After ethical committee approval, full informed consent was obtained from each patient. Inclusion criteria were: lesion size ≥ 1.5 cm², age ≤ 60, chondral defect Outerbridge type III or IV, full rehabilitation protocol compliance, full anamnesis available, signed consent, full surgeon report available. Exclusion criteria were tibiofemoral or patellofemoral mal-alignment, knee instability, kissing lesions, advanced OA, rheumatic arthritis, metabolic or neoplastic diseases. Every patient, after informed consent, was asked to undergo a second look arthroscopy with biopsy for assessing the state of the repair at 12 months follow-up. Every patient was scheduled for a post-operative MRI with a 1.5 Tesla scanner. Failure was defined as the need of a new surgical procedure to treat persisting pain or effusion in the previously operated knee. Patients were retrospectively analyzed with standardized assessment tools such as the IKDC score [27], the Lysholm score [28], the VAS pain score and the Tegner activity scale [29].

2.2. Surgical technique

The CMBMC surgical technique has been described in detail by Gigante et al. [26]. Briefly, for bone marrow harvest, a small area over the iliac crest donor site was draped. A 2.5 mm Jamshidi needle was inserted percutaneously into the iliac crest, sixty ml of bone marrow blood were aspirated and processed with the MarrowStim Concentration kit (Biomet, Warsaw, IN) according to the manufacturer’s instructions, obtaining 3–4 ml of BMC. The PGA–HA matrix was immersed with the BMC and kept until implantation.

After diagnostic arthroscopy to confirm the indication for the procedure (Fig. 1a), the chondral lesion was debrided, measured and microfractures were performed using appropriate awls (Fig. 1b–e). The measured size of the lesion was used to adjust a rubber template to the exact shape of the defect. The PGA–HA matrix was cut to match the defect shape and size. The water flow was stopped and water was aspirated from the joint cavity. A 10:1 mixture of 1–2 ml fibrin glue and BMC was applied to the lesion bed using a long needle (Fig. 1f). The PGA–HA matrix immersed with BMC was inserted through the appropriate portal with a grasper and placed with a probe (Fig. 1g).

Table 1 Baseline characteristics of patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PGA–HA-CMBMC (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at surgical intervention [years]</td>
<td>48 (±9)</td>
</tr>
<tr>
<td>Gender [male, n (%)]</td>
<td>5 (55)</td>
</tr>
<tr>
<td>Localization [MFC, n (%)]</td>
<td>6 (66)</td>
</tr>
<tr>
<td>Number of previous surgeries</td>
<td>0.9 (±0.3)</td>
</tr>
<tr>
<td>Associated pathology [yes, n (%)]</td>
<td>7 (77)</td>
</tr>
<tr>
<td>Correction of pathology [yes, n (%)]</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Lesion size [cm²]</td>
<td>2.6 (±0.5)</td>
</tr>
<tr>
<td>Follow-up [months]</td>
<td>22 (±2)</td>
</tr>
</tbody>
</table>

PGA–HA-CMBMC = polyglycolic acid/hyaluronan-covered microfracture and bone marrow concentrate; MFC = medial femoral condyle.

Fig. 2. Second-look arthroscopy and biopsy harvest. a the Jamshidi needle is inserted from the appropriate portal; b and the bioptic cylinder is harvested. c Second look biopsy showing a repair in level with the surrounding cartilage, completely integrated and with a smooth surface. A fat drop is visible, which is the result of the biopsy harvest. d Repair with incomplete filling (~50%), fissured surface and a large cleft (arrow).

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Table 2
Patient-reported outcome data.

<table>
<thead>
<tr>
<th>Score</th>
<th>PGA-HA-CMBMC (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysolm pre-op.</td>
<td>68 (±10)*</td>
</tr>
<tr>
<td>Lysolm post-op.</td>
<td>88 (±18)*</td>
</tr>
<tr>
<td>IKDC pre-op.</td>
<td>52 (±12)*</td>
</tr>
<tr>
<td>IKDC post-op.</td>
<td>86 (±15)*</td>
</tr>
<tr>
<td>VAS pre-op.</td>
<td>7.4 (±2.3)*</td>
</tr>
<tr>
<td>VAS post-op.</td>
<td>1.5 (±2.7)*</td>
</tr>
<tr>
<td>Tegner pre-injury</td>
<td>4 (4-6)*</td>
</tr>
<tr>
<td>Tegner post-injury</td>
<td>3 (2-3)***</td>
</tr>
<tr>
<td>Tegner post-op.</td>
<td>4 (3.5 ± 6)***</td>
</tr>
</tbody>
</table>

PGA-HA-CMBMC = polyglycolic acid/hyaluronan-covered microfracture and bone marrow concentrate; Lysolm, IKDC and VAS are expressed as mean (±SD); Tegner is expressed as median (interquartile range).

* Pre-op, statistically significantly different from Post-op (t-test).

** Pre-injury statistically significantly different from Post-injury.

*** Post-injury statistically significantly different from Post-op.

(Wilcoxon sum rank test). Post-op refers to the latest follow-up.

Then an additional 2–3 mL of the fibrin glue-BMC mixture were dispersed over the matrix and allowed to solidify for 2–3 min (Fig. 1h).

Finally, excess fibrin glue-BMC was removed and the knee repeatedly flexed and extended to check membrane stability (Fig. 1i).

For rehabilitation, the patients started continuous passive motion (CPM) on day 4–5 and partial weight-bearing at 3 weeks, progressing to full weight-bearing at 6 weeks. Isometric quadriceps and hamstring training and straight leg raises were advised during the non-weight-bearing period. Light sports activities such as swimming, cycling or jogging on even soft ground were allowed at 6 months. Permission to participate in unrestricted sports activity was given after 12 months.

2.3. Second-look arthroscopy

Two patients consented to second-look arthroscopy and biopsy harvest. Three additional patients consented to second-look arthroscopy but did not consent to biopsy. Biopsies were taken with a standard 2.5 mm diameter Jamshidi needle (Fig. 2a, b). The specimens were placed in 10% formalin and sent for histology processing.

2.4. Histology

Histological characteristics of the repair tissue were evaluated. Specimens were decalcified, paraffin-embedded and stained with Safranin-O to detect the presence of glycosaminoglycans. Polarized microscopy was used to discriminate between hyaline-like cartilage and fibro-cartilage. The International Cartilage Repair Society (ICRS) II Histology Scoring System [30] was used to evaluate the quality of the repair tissue. Histological evaluation was performed blindly by two different investigators and scores were averaged.

2.5. Statistical Analysis

The paired t-test was performed for the IKDC score, the Lysholm score and the VAS to compare pre- and postoperative values. Data are expressed as means with standard deviations. The nonparametric Wilcoxon-signed rank test was performed for the Tegner activity scale to compare pre- and postoperative values. Data are expressed as medians and interquartile ranges. For all tests, p < 0.05 was considered significant. The statistical software SPSS (Version 17.0) was used for biometric analysis.

3. Results

3.1. Clinical Outcome

Patients’ characteristics are shown in Table 1. Previous surgeries were: 4 meniscectomies, 3 articular debridement and 1 anterior cruciate ligament (ACL) reconstruction. Concomitant interventions at the time of surgery were 1 ACL calcification removal, 1 osteo-chondral fragment fixation, 3 meniscectomy and 1 trochlear resurfacing. No patient-related or device-related complications were encountered. All patients followed the standardized rehabilitation protocol.

At 22 (±2) months follow-up, patients treated with PGA-HA-CMBMC showed significant (p < 0.05) improvement in IKDC subjective score from 68 pre-operatively to 88 post-operatively, in Lysholm score from 52 to 86 and in VAS pain score from 7.4 pre-operatively to 1.5 post-operatively (Table 2). The Tegner activity scale showed no significant difference from pre-injury (4) to post-operative levels (4) at latest follow-up, but significant improvement in the activity level from post-injury (3) to post-operative activity levels (4).

The procedure failed in one patient, who needs a re-operation due to persisting pain. The patient (latest VAS = 8) was subjected to second look arthroscopy that showed the persistence of the defect at the medial femoral condyle. This female patient, with a body mass index (BMI) of 33, is currently losing weight in order to undergo a new surgical intervention.

![Image](https://example.com/image.png)

Fig. 3. Postoperative MRI scans representative of the average quality of cartilage repair. a, b The T1 coronal and sagittal sections (10 months post-operatively) of the left knee show complete defect filling of the lateral compartment defect (arrows), isointense cartilage signal with small hypointense spots and subchondral bone irregularity. Moderate marrow oedema was visible with T2 sequences (not shown). The patient (45 y at the time of the procedure) had previously undergone a shaving procedure.

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At the time of the second-look arthroscopy (Fig. 2) all the patients but one were asymptomatic. According to the IKDC CBA evaluation, 1 out of 5 patients treated with PGA–HA–CMBMC was graded normal, 3 nearly normal (median 10, range 7–12). The patient scoring 7 was the one that failed (Fig. 2d).

Four MRIs were performed with an average of 10 ± 1.6 months follow-up (range 8–12 months). All patients showed complete defect and volume filling with resurfacing of the articular cartilage to the original cartilage level (Fig. 3a, b, white arrows). Mild bone marrow oedema and some subchondral irregularities were observed in all cases. Non-homogeneous cartilage signal was observed in 2 out of 4 cases; fissures were noted in 1 case, surface irregularities in 1 case and a slight hypertrophy of the repair tissue was observed in 1 case.

3.3. Histological evaluation

Biopsies were obtained from two patients (Fig. 4). Safranin O staining showed that the repair tissue was rich in proteoglycan and chondrocytic cells. In line with nearly normal MRI findings and improvement in clinical scores, the biopsy proofed hyaline-like repair tissue formation after the implantation of the PGA–HA matrix immersed with autologous BMC (Fig. 4a). The repair tissue formed in the patient with the failed treatment was rich in chondrocytes but thin and of a fibrocartilaginous appearance (Fig. 4b). There were no remnants of the PGA–HA matrix and no signs of foreign body reaction or necrosis. According to the IKDC II score they scored respectively an overall of 93 and 41, with a tissue morphology of 100 and 30.

Detailed patients’ baseline characteristics and outcomes are shown in Table 3.

4. Discussion

The most important finding of the present study is that, with an average follow-up of 22 months, the PGA–HA–CMBMC technique is safe and effective in improving symptoms of patients affected by focal condylar cartilage lesions, and that the PGA–HA matrix has the potential to induce hyaline-like cartilage repair tissue in microfracture.

In recent years one-step cartilage repair procedures have evolved that target to treat chondral knee defects and to improve the microfracture procedure [17,18,31–33]. All these approaches have in common that the microfracture procedure is used to allow progenitor cells to enter the defect. The diverse procedures differ in the type of matrix that is used to cover the defect, the augmentation with autologous blood derivatives and the surgical technique, including collagen matrices or PGA–HA matrices, the addition of platelet-rich plasma (PRP) and the use of all arthroscopic or mini-open procedures [15,17,18,31–34].

Behrens described the original AMIC (autologous matrix-induced chondrogenesis) technique with the use of a porcine collagen type I/III membrane for covering of the microfractured defect and the injection of fibrin mixed with autologous serum underneath the membrane for the treatment of chondral defects [14,35]. Gille et al. treated large (mean 4 cm²) chondral defects with AMIC and found significant clinical improvement at an average of 37 months follow-up. However, the quality of the regenerated tissue and the level of tissue filling were not ideal with approximately 1/2 of the MRI scans showing incomplete defect filling and subchondral bone abnormalities [15].

In a retrospective cohort study, Kusano and colleagues reported largest clinical improvement in patients treated for osteochondral defects with the AMIC procedure, while defects in the patellofemoral joint and on the femoral condyle showed less improvement. In addition, half of the patients treated for patellar defects required mobilization under anesthesia due to knee stiffness, tissue regeneration was apparently variable and MRI scans revealed some complete filling, some empty defect and some hypertrophic cartilage repair tissue [33]. In a prospective study, Efe et al. used a type I collagen gel for the treatment of small (1 cm²) cartilage lesions. The technique did not use the microfracture approach and relied on chondrocyte migration from the surrounding healthy cartilage. The authors reported good clinical results as assessed by IKDC score, Tegner activity scale and the VAS pain score as well as MRI improvements with complete defect filling at up to 2 years follow-up [32]. Siclari et al. treated tibial and femoral cartilage defects in 52 patients with subchondral perforations made by drilling and a PRP-augmented PGA–HA matrix.

The authors reported a significant and clinical meaningful improvement at 12 months follow-up as assessed by the Knee injury and Osteoarthritis Outcome Score (KOOS) [18]. Dhollander et al. reported on a pilot study with five patients using microfracture and a PGA–HA matrix enriched with autologous serum. The authors observed noticeable clinical improvement; however, MRI scans revealed different percentages of incomplete filling, subchondral bone irregularities,
subchondral cysts and intralesional osteophytes [34]. The same group analyzed another five patients treated with the original AMIC technique combined with a PRP gel. Again, the favorable clinical outcomes were not matched by MRI improvements. At 2 years follow-up, the authors reported persistence of subchondral bone abnormalities, incomplete filling or hypertrophy of the repair tissue and intralesional osteophyte formation [31].

In the present pilot study, the application PGA–HA-CMBMC led to a significant improvement in all the analyzed clinical assessment tools from baseline to the latest follow-up at 22 months. MRI scans revealed the persistence of bone marrow oedema and subchondral plate irregularities, but also showed a complete defect fill in all the cases. These good clinical results were obtained in a challenging patient group with advanced age, and multiple previous and concomitant surgical procedures. In particular a higher age is considered to be critical in microfracture. Kreuz and colleagues found better clinical and MRI outcomes at 3 years follow-up in patients younger than 40 years. Patients older than 40 years showed improvement as assessed by the ICRS scoring at follow-up compared to the pre-operative situation, but the scores deteriorated between 1.5 years and 3 years after the surgery [36]. It has to be highlighted that the average lesion size treated in this study, between 2 and 3 cm², was small and could have been treated with success with microfracture. However, the good outcome possibly obtained with microfracture has been shown to potentially decrease with time [36]. Therefore it has been hypothesized that adding BMC and a covering membrane could have been helpful in the present group of patients.

Moreover, the results obtained with PGA–HA-CMBMC after 22 months may be promising for a good future outcome, since in ACI the patient status at two years of follow-up is considered as an important indicator [37].

One obese risk patient with a BMI of 33 (1 out of 9 patients, 11%) required a successive surgical intervention for persistence of pain in the knee. This or even a higher percentage of reoperations must be expected when performing cartilage repair procedures [31,33,34]. For instance, in ACI, revision surgeries between 0% and 49% [38,40] have been reported, while graft failures may occur in 5% to 13% of the cases [39,41].

Only a few studies have investigated the histological outcomes of one-step procedures in the treatment of articular cartilage lesions. Giannini and colleagues reported the use of BMC and PRP gel with a hyaluronic acid-based membrane or a collagen powder to treat talar osteochondral lesions. In this study a functional improvement was observed for all the patients, and 3 biopsies showed different degrees of tissue remodeling toward hyaline-like cartilage [42]. Siclari et al. performed 10 second look arthroscopies and harvested 5 biopsies. The repair tissue was of a tough condition, appeared whiter than the surrounding cartilage and a certain degree of surface irregularity and an asymptomatic hypertrophy was observed. Histological evaluation uniformly showed hyaline-like cartilage repair with good subchondral integration [18]. In the present pilot study, on average, a nearly normal macroscopic appearance of the cartilage repair tissue was found according to ICRS CRA. Histological evaluation of two biopsies revealed one hyaline-like cartilage repair tissue formation and one fibrocartilaginous tissue formation in the risk patient that needed re-operation. Although statistically not relevant, the fact that one out of two patients showed hyaline-like repair tissue formation may be promising if compared to the previously reported results for ACI and ACI-related procedures [43,44].

This indicates that cells derived from autologous BMC and seeded on a scaffold may differentiate into mature chondrocytes or may stimulate subchondral progenitor cells released by the microfracture procedure to produce a cartilaginous repair tissue when applied in human adult articular cartilage lesions. These clinical observations may confirm recent in vitro results that demonstrated that human MSCs from bone marrow aspirate can proliferate on collagen scaffolds

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Detailed patients’ baseline characteristics and outcome data.</th>
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<tbody>
<tr>
<td>Patient</td>
<td>Age</td>
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<td>1</td>
<td>45</td>
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<tr>
<td>2</td>
<td>37</td>
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<td>3</td>
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<td>48</td>
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<tr>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
</tr>
</tbody>
</table>

ICRS CRA = International Cartilage Repair Society – cartilage repair assessment; MFC = medial femoral condyle; LFC = lateral femoral condyle; TR = trochlea; ACL = anterior cruciate ligament.

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and differentiate into chondrocytes without growth factor supplementation [45]. The mean age of the study population was 48 ± 9 years (range 37–60). Therefore it is likely that some degree of degenerative changes occurred at least in some of the patients. Although osteoarthritic defects are in general not or hardly indicated for current cartilage repair techniques, ACI and scaffold-assisted ACI procedure have been shown to have the capability to improve the symptoms in patients with early osteoarthritis and may postpone the need for prosthetic replacement [7,46,47]. However, if compared to original ACI, one-step procedures are relatively inexpensive and have been used in older patients with radiologically confirmed degenerative changes (up to 65 years-old) providing pain relief and good histological results [18]. In addition, the PGA–HA matrix can be cut to the size of the defect and can be securely fixated by glue as shown in this study as well as by cartilage suture, trans-osseous suture or pin/nail fixation [17,48]. Biomechanical in vitro studies have shown that covering a cartilage defect with the PGA-based matrix restores the joint compression forces toward forces found in normal joints [49]. Therefore, the textile and mechanically stable felt-like structure of the PGA–HA matrix may be favorable for arthroscopic approaches and for the treatment of degenerative defects that lack an intact cartilage rim. However, further clinical studies involving more degenerative and/or osteoarthritic defects are needed, before the use of such approaches can be recommended unrestrictedly to this patient group.

It has to be highlighted that the procedure detailed in this pilot study and the other “one-step” procedures have been introduced just recently in the clinical practice. To date, the potential to maintain high subjective outcomes at long follow up, the potential to avoid or slow down the onset of osteoarthritis and in general the real benefit for the patient has still to be proven against less expensive procedures such as microfracture. In this regard, randomized controlled trials versus microfracture and/or versus MACI would be highly beneficial.

Limitations of this study are small sample size, short-term follow-up and lack of control group. In addition, the patients were not stratified for presence of early OA with preoperative plain X-ray. The strength of the present study is that isolated condylar lesions of similar size were treated in absence of limb malalignment and major associated concomitant conditions.

Conflict of interest

CK is employee of TransTissue Technologies GmbH (TTT) and consultant of BioTissue AG. The other Authors have no conflicts to report.

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