STABLE CELL-FREE 3D CARTILAGE IMPLANT
BioTissue, a double spin-off of the Charité University Hospital in Berlin and the University Hospital in Freiburg, is the pioneer par excellence in the field of regenerative cartilage treatments.

The BioTissue products combine the most advanced technology of regenerative medicine with the latest scientific developments in the field of scaffold and stem cell research, in order to satisfy the growing demands for biological treatment options in orthopaedics. This has been achieved by the continuous cooperation between BioTissue’s own Research & Development department and an international key opinion leader network.

BioTissue’s specific know-how in the field of biological scaffold technologies has led to the development of a unique 3D scaffold for the optimal differentiation of mesenchymal cells. The combination of BioTissue’s patented scaffold technology with standard marrow stimulation techniques for the treatment of cartilage lesions, such as microfracturing or Pridie drilling, enables easy and excellent surgical handling and leads to optimal repair tissue formation.

This scaffold has been tested in numerous preclinical and clinical studies and has been proven to be an ideal therapeutic option for traumatic and degenerative cartilage defects supporting hyaline-like to hyaline cartilage formation.

The fulfilment of all regulatory requirements, the intensive exchange with international key opinion leaders and the highly qualified BioTissue team have brought forth another innovative product for the treatment of joint cartilage defects:

BioTissue’s one-step procedure product chondrotissue®
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>chondrotissue® product highlights</td>
<td>6</td>
</tr>
<tr>
<td>Scientific background</td>
<td>8</td>
</tr>
<tr>
<td>Clinical data</td>
<td>10</td>
</tr>
<tr>
<td>Safety</td>
<td>23</td>
</tr>
<tr>
<td>Indications</td>
<td>25</td>
</tr>
<tr>
<td>Surgical technique</td>
<td>26</td>
</tr>
<tr>
<td>Rehabilitation</td>
<td>29</td>
</tr>
<tr>
<td>Scientific publications</td>
<td>30</td>
</tr>
</tbody>
</table>
PRODUCT HIGHLIGHTS

Implant

- is based on a patented 3D scaffold consisting of two components:
  1. a highly porous textile polyglycolic acid felt ensuring mechanical stability
  2. hyaluronic acid for optimal hyaline-like repair tissue formation
- dimensions: 20 x 30 x 1 mm
- does not contain any components of animal or human origin
- is indicated for traumatic and focal degenerative cartilage defects (Outerbridge grade III to IV) in synovial joints
- is CE marked and manufactured according to the European Medical Device Directive and GMP standards

Application

- one-step procedure and off-the-shelf ready-to-use implant
- in combination with marrow stimulating techniques such as microfracturing or Pridie drilling
- implantation by arthroscopy or arthrotomy
- stable subchondral fixation
- no intact surrounding cartilage is required, leading to extended indication

Performance and Data

- optimal defect covering and defect filling
- correction of abnormal biomechanical forces in the defect area (capping effect)
- resorption after approx. 4-6 months after implantation
- hyaline-like cartilage formation (proven by histologies)
- pain reduction
- increased patients' mobility and quality of life

over 4500 patients treated successfully
chondrotissue® is a very smart cartilage implant for one-step cartilage repair procedures.

It has got unique mechanical characteristics and provides a “capping effect” in the defect area, as approved by biomechanical tests. This means that irregular biomechanical forces in the defect zone will be corrected to the normal situation after implanting chondrotissue®.

This “capping effect” avoids immediately further cartilage deterioration in the surrounding cartilage. chondrotissue® can be implanted arthroscopically. It is an easy and fast procedure by using resorbable nails.

Based on my experience, chondrotissue® improves the results of marrow stimulating techniques, provides high quality hyaline cartilage formation - as proven by MRI and histologies - and leads to significant improvement of the clinical symptoms, as assessed by standard cartilage scores.

Mirco Herbort, MD
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Clinic for Traumatology, Hand Surgery and Reconstructive Surgery
Matrix-induced chondrogenesis

**SCIENTIFIC BACKGROUND**

**MICROFRACTURING WITHOUT CHONDROTISSUE®**

1. Marrow stimulation (microfracturing, Pridie drilling)
2. Bleeding and cell migration
3. Formation of a fibrous repair tissue

Marrow stimulating techniques such as microfracturing or Pridie drilling are frequently used to treat cartilage defects.

Though they might help for very small defects without weight-bearing, clinical outcomes have shown that they are ineffective for the treatment of medium to bigger defects in the long term follow-up. They lead to fibrous repair tissue that will degenerate again under the effect of compression and shear forces.

Thus, a **stable 3D scaffold** is needed to:
1. Correct disturbed biomechanical forces in the defect area (capping effect)
2. Home and keep in place migrating mesenchymal stem and progenitor cells
3. Trigger the formation of hyaline-like cartilage

**MICROFRACTURING WITH CHONDROTISSUE®**

1a. Application of chondrotissue®
2a. Migration of stem and progenitor cells into chondrotissue®
3a. Formation of a hyaline-like repair tissue

By covering the defect area with chondrotissue® after bone marrow stimulating methods, mesenchymal stem and progenitor cells migrate into and remain in the scaffold. Cells find an optimal stable 3D environment to form hyaline-like repair tissue, as confirmed by the histological data provided on following pages.

The application of chondrotissue® improves the results obtained after standard bone marrow stimulation techniques by enhancing the formation of hyaline-like repair tissue, compared to microfracturing.
RESORBABLE POLYMER-BASED TEXTILE SCAFFOLD

- guarantees initial mechanical stability
- provides opportunity for stable fixation
- allows optimal 3D cell distribution
- offers an environment for mesenchymal cells

HYALURONIC ACID

- is a natural polymer with important functions in the knee joint
- provides an important stimulus for chondrogenic cell differentiation and the subsequent formation of a hyaline cartilage matrix
- contributes to the visco-elasticity of the cartilage and protects against friction and impact loading
In a prospective study, 52 patients underwent arthroscopic implantation of chondrotissue® immersed in platelet-rich plasma (PRP) for treating full-thickness articular cartilage knee defects.

It was investigated whether chondrotissue® immersed in PRP improves patients outcome in the Knee Injury and Osteoarthritis Outcome Score (KOOS) with up to 5 years of follow-up.

Patients who received chondrotissue® improved significantly in median KOOS as early as 3 months after surgery ($p < 0.001$) and at 6, 9 and 12 months ($p < 0.001$ each) postoperatively (Fig. 1 A-E).

The median KOOS subcategories pain and symptoms improved from preoperatively to last follow-up: 55 to 91 ($p < 0.001$) and 57 to 88 ($p < 0.001$), respectively.

Compared to the time before surgery, patients showed improvements in activities of daily living (69 to 86; $p < 0.001$), sports and recreation (36 to 70; $p < 0.001$) and knee-related quality of life (38 to 73; $p < 0.001$) at 12 months postoperatively.

All KOOS subcategories showed improvement at three months postoperatively compared to the KOOS before surgery and this improvement increased continuously for up to 12 months.

In summary, chondrotissue® combined with PRP improved patient-reported outcomes (KOOS) during the 12 months follow-up.
At 2-year follow-up, the situation of the previously described 52 patients (mean age: 44 years) was assessed again using the Knee Injury and Osteoarthritis Outcome Score (KOOS) and compared to the preoperative situation and to the situation at 3-24 months follow-up.

After 2-year postoperatively, there were no clinical signs of persistent knee joint infections or inflammations, allergic reactions, foreign body reactions nor knee joint effusion or swelling. Temporary blocking and signs for ablation of the implant or loosening of the newly formed repair tissue were not evident.

Patients who received the PRP-enriched chondrotissue® showed a significant improvement (p<0.001) in all KOOS subcategories at 3-, 12- and 24-month follow-up, compared to the preoperative situation (Fig. 2).

At 12- and 24-month follow-up, clinical scores significantly improved compared to the scores obtained at 3-month follow-up. There was no difference between the KOOS at 12-month follow-up and 24-month follow-up. The overall KOOS was in mean 50.3 at baseline and increased to in mean 82.9 at 2-year follow-up.

In summary, the treatment with PRP-enriched chondrotissue® improved clinical outcome (KOOS) during 24 months of follow-up. The clinical results obtained at 2-year follow-up confirmed the good findings at 1 year follow-up.

Figure 2: Clinical evaluation was performed using the KOOS. A KOOS of 100 indicates no knee problems and a KOOS of zero indicates severe knee problems.

Clinical scoring and analysis of KOOS values 5 years after surgery showed a clinically meaningful (ΔKOOS > 10) and significant (p < 0.001) improvement in all KOOS subcategories, compared to the preoperative situation (Fig. 3).

Pain improved from in mean 54.1 preoperatively to in mean 93.5 at 5-year follow-up, symptoms from 56.3 to 91.3, activities of daily living from 68.1 to 89.5, sport & recreation from 35.5 to 74.5, and the subcategory quality of live improved from 37.2 to 76.4 at 5-year follow-up.

The overall KOOS was in mean 50.3 at baseline and increased to in mean 85.0 at 5 years follow-up, leading to an average ΔKOOS of 34.7.

Subgroup analysis (Fig. 4) showed that there were no differences in clinical outcome regarding degenerative condition of the knee, defect size and location, gender, age as well as body mass index.

In summary, the treatment with PRP-enriched chondrotissue® significantly improved clinical outcome (KOOS) during 5 years of follow-up. Covering of focal cartilage defects with the chondrotissue® implant and PRP after bone marrow stimulation leads to a long lasting and stable improvement of the patients’ situation.

The clinical and histological results at 5-year follow-up confirm the good findings at 1 year and suggest a good long-term outcome.

Figure 4: Subgroup analysis of the clinical outcome after five years as assessed by KOOS. Patients’ KOOS data were stratified according to the degenerative status of the operated knee (Kellgren-Lawrence grading 0-1 versus Kellgren-Lawrence grading of 2-3), defect size (≤ 3 cm² versus > 3 cm²), defect localization (femoral versus tibial localization), gender (females versus males), age (≤ 45 years versus > 45 years) and BMI (< 25 versus ≥ 25).
MRI Evaluations

**Figure 5 A-B**: The preoperative MRI of a 43-year old female patient revealed a 0.7 cm²-big cartilage defect of the lateral talus (A). 7 months after the implantation of chondrotissue® the MRI showed perfect defect filling and excellent tissue integration to the surrounding cartilage (B).¹

**Figure 6 A-B**: Intraoperative arthroscopy of a 54-year old female patient showed a 3 cm²-big degenerative cartilage defect of the medial femoral condyle (A). At 12 months follow-up the defect was completely filled with new cartilage tissue. The repair tissue was characterized by optimal integration to the adjacent cartilage and a smooth surface. Only both resorbable nails used for fixation were still visible.²

Clinical data following treatment with chondrotissue® show excellent cartilage repair, high volume defect filling, and smooth peripheral integration of the repair tissue to the adjacent tissue.
**Figure 7 A-B:** Intraoperative arthroscopy of a 35-year old male patient revealed a 4 cm²-big traumatic cartilage defect of the lateral femoral condyle (A). 12 months after the implantation of chondrotissue® the MRI showed optimum defect filling and a homogeneous repair tissue with smooth surface and good integration into the surrounding cartilage and the subchondral bone (B).

**Figure 8 A-B:** MRIs 4 years after implantation of chondrotissue® immersed in platelet-rich plasma (PRP)
The MRI showed good defect filling with repair tissue of iso- to hyperintense signal compared to the adjacent cartilage (A). Some bone signal was evident (B).

1. Bergmann et al. 7 months clinical follow-up after arthroscopic implantation of chondrotissue in a cartilage lesion of the lateral talus.

2. Zantop et al. Arthroscopic implantation of a matrix to cover large chondral defect during microfracture.

Figure 9 A-B: Repair tissue biopsy 5 months after surgery
Positive Alcian blue staining of proteoglycans in a chondrotissue® biopsy, surface of the repair tissue (A asterisk), central part (A diamond) and bone/cartilage-interface (B black arrow)⁴

Figure 10 A-B: Repair tissue biopsy 9 months after surgery
Positive collagen type II staining of a chondrotissue® biopsy, surface of the repair tissue (A asterisk), central part (A diamond) and bone/cartilage-interface (B black arrow)⁴

Figure 11 A-B: Repair tissue biopsy 18 months after surgery
Hematoxylin & eosin overview staining of a repair tissue biopsy showed areas with round chondrocytic cells (A black arrow) and cells in a chondron-like formation (B white arrows)⁵
Figure 12 A-D: Repair tissue biopsy at 9 months after chondrotissue® implantation immersed with PRP

Histological evaluation using hematoxylin & eosin staining showed homogeneous repair tissue with round chondrocytic cells (A), good integration of the repair tissue to the underlying subchondral bone (B) and to the native adjacent cartilage (C). Immuno-histochemistry of the central biopsy region showed the formation of intracellular and extracellular type II collagen (D), indicating hyaline-like cartilage tissue.⁶

Histological evaluation shows a hyaline-like cartilage repair tissue, round chondrocytic cells, a proteoglycan-rich matrix, and collagen type II. After the chondrotissue® treatment the repair tissue is firmly integrated to the subchondral bone and shows a homogenous hyaline-like structure.

⁴ Behrendt unpublished data

⁵ Patrascu et al. Repair of a post-traumatic cartilage defect with a cell-free polymer-based cartilage implant: a follow-up at two years by MRI and histological review.

⁶ Siclari et al. A cell-free scaffold-based cartilage repair provides improved function hyaline-like repair at one year.
Figure 13 A-L: Repair tissue biopsy at 18-24 months after chondrotissue® implantation immersed with PRP

Histological evaluation of the biopsies using hematoxylin & eosin staining showed repair tissue, rich in round-shaped cells with a chondrocytic appearance and a rough, well structured and smooth extracellular matrix (A-D). There were no remnants of the chondrotissue® implant and no signs of foreign body reaction or necrosis. Alcian blue staining showed that the repair tissue was rich in proteoglycans and chondrocytic cells (E-H). Immuno-histochemical anti-collagen type II staining showed the presence of collagen type II in the extracellular matrix (I-L) and confirmed hyaline-like to hyaline repair tissue formation at 18–24 months after implantation of the chondrotissue®.

Figure 14 A-F: Repair tissue biopsy at 3 years after chondrotissue® implantation in a 3-year old child

Biopsies of the child’s talar cartilage were taken from the central dome, where chondrotissue® had been implanted, and from the talar neck (normal cartilage) at 3 years after injury.

Histological evaluation revealed a cartilage matrix rich in cells with a homogenous cell distribution and a columnar cell arrangement of the chondrocytic cells (A-C). The repair tissue was a little less structured but generally showed a very similar appearance compared to the native cartilage tissue sample (D-F).

In contrast to fibrous cartilage repair tissue, no fibroblasts, a homogenous cartilage-bone-interface and a hyaline-like to hyaline structure was examined (A). The repair tissue showed a for hyaline-like cartilage characteristic formation of collagen type II (C) and proteoglycans (B) in the central and lower part of the biopsy. There were no visible chondrotissue® scaffold residues.

Mason et al. The use of a cell-free chondroinductive implant in a child with massive cartilage loss of the talus after an open fracture dislocation of the ankle: a case report.
Another study investigated the effect of perpendicular and 30°-tilted nail fixation to the matrix surface on the joint compression forces. In a porcine knee model, joint compression forces were recorded with a digital pressure sensor above the medial meniscus and with axial compression of 100 N by the use of a material testing machine. The forces were recorded for an intact femoral condyle as well as for standardized 25 x 20 mm cartilage defects covered with chondrotissue® and fixed by conventional suture technique or nail fixation with a biodegradable nail, which was performed perpendicularly or 30° tilted to the matrix surface.

Figure 15:
Box-plot diagram of results of peak contact pressure in all tested groups.

Results in knees with cartilage defects showed (Fig. 15) that the peak compression forces (mean, 824 ± 33 kPa) were significantly increased compared to the intact knee joint (564 kPa). After implantation of the chondrotissue® scaffold with a chondral suture (581.3 kPa) or perpendicular nail fixation, the joint compression forces of the cartilage defect were significantly decreased (630.7 kPa). There were no significant differences compared to the intact knee. In contrast to that, 30° tilted nail insertion showed significantly increased (1,740 kPa) mean joint compression forces (Fig. 16 A-B).
The insertion of chondrotissue® into the cartilage defect restores joint compression forces to the level of intact cartilage conditions.

For nail insertion, a perpendicular angle is needed to avoid increased joint compression forces. Covering of the defect with the chondrotissue® scaffold reduced the compression forces in the knee joint comparable to levels of an intact knee (Fig. 17). These data suggest that the implantation of chondrotissue® corrects increased compression forces in the defect that can lead to decreased forces and pathological effects in the surrounding cartilage.

In summary, this study showed that cartilage defects lead to increased compression forces compared to a healthy knee.

Herbert et al. Arthroscopic fixation of matrix-associated autologous chondrocyte implantation: importance of fixation pin angle on joint compression forces.
chondrotissue® is based on a resorbable polyglycolic acid scaffold and hyaluronic acid. Both components are 100% non-animal origin and bio-compatible.

chondrotissue® is manufactured in a high-tech cleanroom-lab according to the European Medical Device Directive and GMP (Good Manufacturing Practice) standards.

Every chondrotissue® lot is subjected to strict quality control tests before it is released to the market.

As a CE marked class III medical device chondrotissue® and its manufacturer BioTissue are yearly subjected to strict audits by the Notified Body.
INDICATIONS

- chondral traumatic and focal degenerative cartilage defects of synovial joints
- grade III and IV of the Outerbridge scale
- for synovial joint cartilage defects of the e.g. knee, foot, ankle, hip, shoulder etc.
- can be used when a bone marrow stimulating technique, such as microfracturing or Pridie drilling, is performed
The implantation of chondrotissue® can be done in only one step: there is no need to take a cartilage biopsy from the patient and no need to wait weeks for cell culture.

1 Debridement
The cartilage defect must be debrided until the subchondral bone is exposed. Damaged cartilage must be removed and the defect must be prepared.

2 Measurement
The debrided defect should be measured with an arthroscopic measuring gauge or with a simple surgical ruler if surgery is performed by arthrotomy.

3 Bone marrow stimulation
The defect should be perforated with an awl or a drill with spacing around 3-5 mm.

4 Reconstitution of chondrotissue®
In order to restore the elastic properties of chondrotissue®, it is recommended to put it directly in the sterile container supplied with 2-3 ml of one of following sterile rehydrating fluids for approx. 2 minutes.
Following alternatives shall be used to reconstitute chondrotissue®:

- **human autologous platelet-rich plasma (PRP)**
- **physiological saline for infusion**
- **autologous human serum** taken before implantation and prepared from approx. 9 ml of blood. The blood can be centrifuged for 10 minutes or left to stand at room temperature for about 30 minutes until the blood clot has settled.
  IMPORTANT: please use serum monovettes, not EDTA monovettes.

If too little PRP or human serum is available, physiological saline for infusion can be supplemented to reconstitute the elasticity of chondrotissue®.

5 Preparation of chondrotissue®
The moistened chondrotissue® can then be cut exactly to size to fit the defect.

6-7 Fixing chondrotissue®
chondrotissue® can be fixed in the defect using common orthopaedic fixation methods e.g.:

- **bioreabsorbable nails** (e.g. SmartNail™ from ConMed Linvatec)
- **transosseous ancher knot fixation**
- **cartilage suture**
- **fibrin glue**: Please apply the glue to the edges of the chondrotissue® - previously placed in the defect - and distribute it evenly.

Please read the package leaflet for more information concerning the product and its use.
REHABILITATION
The information given below is intended only as a recommendation and depends on the size and the site of the defect, the patient’s age and the general demands of daily living.

**Femoral and tibial defects**

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2-6</th>
<th>After 6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading / Mobilisation</td>
<td>Foot sole contact with walking support/Braces</td>
<td>Foot sole contact with walking support/ Femoral condyle – CPM* with restriction Week 2 - 3: 0/0/60° Week 4 - 6: 0/0/90°</td>
<td>Increase of loading to full body weight after two weeks/ Free mobility (pain-related restriction)</td>
</tr>
<tr>
<td>Walking, sport</td>
<td>Mobilisation</td>
<td>Aqua gymnastics, swimming</td>
<td>After 6 months: jogging, aqua jogging After 6 - 12 months: skiing After 8 weeks: cycling After 18 months: contact sports</td>
</tr>
</tbody>
</table>

**Patellar and trochlear defects**

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2-7</th>
<th>After 7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobilisation</td>
<td>Braces</td>
<td>CPM* with restriction Week 2 - 3: 0/0/30° Week 4 - 5: 0/0/60° Week 6 - 7: 0/0/90°</td>
<td>Increase of loading to full body weight after two weeks/ Free mobility (pain-related restriction)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Week 1-2</th>
<th>Week 3-4</th>
<th>After 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading</td>
<td>Foot sole contact with walking support</td>
<td>50% body weight with walking support climbing stairs only with healthy leg</td>
<td>Increase of loading to full body weight after two weeks</td>
</tr>
</tbody>
</table>

*CPM : continuous passive motion
SCIENTIFIC PUBLICATIONS


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