BioSeed®-C Guidelines

What is BioSeed®-C?
A unit of BioSeed®-C consists of a support matrix available in various sizes whose length, width and height measure 30 x 20 x 2 mm, 30 x 30 x 1.1 mm, 30 x 20 x 1.1 mm or 50 x 20 x 1.1 mm. This support matrix contains interconnecting cavities in which 28.8 million autologous (body’s own) cells are evenly distributed by means of fibrin adhesive. The autologous chondrocytes are isolated from a cartilage biopsy of the patient and proliferated during a 21-day (+3 days) cultivation phase under GMP-conditions. In the last phase of cultivation, the chondrocytes are embedded in the support matrix together with the fibrin adhesive and then re-applied to the patient.

Composition of the support matrix
The support matrix consists of resorbable polymers on a polyglycolic acid basis. These polymers consist of pure polyglycolic acid or the compound material Polylactin 910 and Poly-p-dioxanone: both original materials are bound together by means of a thermoplastic process without the addition of foreign matter or additives. A support matrix-like material is produced, the pore size of which permits the growth of cells and autologous tissue. The colourless material is soft, elastic in volume and flexible, and can be cut without fraying.

Description of the gelatinous matrix
The main ingredient is fibrin. The fibrin adhesive used is “Tissucol Duo” or “Tisseel”.

Areas of application
The prerequisite for the success of the method is a definite diagnosis. This is only possible through arthroscopy.
Indications for treatment with BioSeed®-C are traumatic and focal chondral and osteochondral defects (Grade III to V according to Outerbridge). Deep bony lesions require spongiosaplasty before transplantation.

Precautions for use
Only use after careful consideration of the benefits and risks for the following cases:
Patients over 60 years of age, cartilage defects of the head of the tibia, advanced arthritic changes, extensive cartilage wear in the medial or lateral compartment or the patellofemoral joint, cartilage damage in connection with osteoarthritis, incorrect leg axes, e.g. varus or valgum abnormalities and condition after meniscectomy.

Contraindications
BioSeed®-C is contraindicated with known hypersensitivity to bovine protein, penicillin, amphotericin B, gentamycin or any constituent of the products. Although no antibiotics are added to the end product, it can contain slight traces of penicillin and amphotericin B and very slight traces of gentamycin, as these substances are initially contained in the biopsy transport and the culture media being used. Further criteria for exclusion are the presence of metabolic, inflammatory, neo-plastic or immunological diseases, as well as complex knee instability. All of these are to be investigated and controlled in advance.

Side effects
Existing hypersensitivity to bovine protein (aprotinin), to penicillin or amphotericin B, gentamycin and/or other components of BioSeed®-C or Tissucol Duo or Tisseel may result in allergic or anaphylactic reactions. The introduction of the support matrix structure causes, as with any foreign substances, a transient tissue reaction. As with all foreign substances a pre-existing infection can be adversely affected.
In view of the possible heparin content of BioSeed®-C – as a result of the fibrin adhesive – exceptional, severe antibody-dependent thrombocytopenia (Type II) with thrombocyte values below 100,000/µl or a rapid reduction to less than 50% of the original value cannot be excluded. With non-sensitive patients the reduction in thrombocytes as a rule starts 6 – 14 days after the start of treatment; with sensitive patients under certain circumstances it can start within hours.

1 For further information please see specialist information on "Tissucol Duo" or "Tisseel". Tissucol Duo and Tisseel are trademarks of Baxter AG
Warnings
BioSeed®-C is made using Tissucol Duo or Tisseel. With the application of medications made from human blood the transmission of infectious diseases as a result of the transmission of pathogens – also as yet of unknown nature – cannot be completely excluded. Consequently the donors are selected according to strict criteria, the plasma donations are tested and selected and the plasma pools are checked. The production process includes measures to isolate and render inactive any viruses.

For the production of Tissucol Duo and Tisseel exclusively plasmas from healthy donors are used, who have been tested negative for antibodies to HIV Type 1 and 2 and the hepatitis-C-virus (HCV) as well as hepatitis-B-virus surface antigen (Hb,Ag). The liver enzyme value (GPT) must not exceed the accepted limit. Also a sample from the plasma pool will be tested for HIV- and HCV antibodies as well as for Hb,Ag and an additional test will be carried out for virus genetic material of HIV Type 1 and 2, HBV and HCV with the polymerase chain reaction (HIQ-PCR).

The polymerase chain reaction (PCR) is a highly sensitive method, which, in contrast to antibody testing, allows direct proof of virus genetic material (virus genome). Only plasma pools in which no virus genetic material of these viruses can be traced are released for further processing.

In the production of Tissucol Duo and Tisseel various process steps are carried out to detect and render inactive infectious pathogens, which include a product-specific steam treatment.

Interactions
Because of possible denaturing, BioSeed®-C should not come into contact with solutions containing alcohol, iodine or heavy metals, which may be contained for example in solutions for disinfection.

BIOSEED®-C TREATMENT PROCEDURE

1. Prior to the biopsy

1.1 Ordering the biopsy kit
You receive one biopsy kit free of charge for each patient. The kit must be ordered in good time before taking the biopsy from our Customer Service Department under the following phone number:

+ 49 (0) 761 7676 –400 / –580

Just-in-time delivery
The date of taking the biopsy and of the transplantation can be discussed when ordering the biopsy kit.

Biopsy kit
The biopsy kit consists of the following components:

- Forms/documents:
  - Biopsy report & production order
  - Patient consent form
  - This product information and guidelines
- Sterile biopsy tube \(\textit{in insulating container}\) with transport medium, supplied in a secondary tube.
- Cardboard box as outer packaging with foam padding
- Stainless steel insulating container
- Medicool-Pack \(\textit{in the insulating container}\)
- Blood Monovettes (for Germany: 12, for other EU countries and Switzerland: 14 Monovettes).
  On delivery, the Monovettes are located in the cut-outs of the foam padding.
- Blood sampling system (cannulas, butterflies, adapters).
- Patient identification labels

NB: Please only use the Monovettes supplied by BioTissue for taking the blood samples. Serum or full blood that reaches us in other containers cannot be used to produce an autologous product in the GMP-laboratories of BioTissue Technologies GmbH.
1.2 Storing the biopsy tube

<table>
<thead>
<tr>
<th>Storage of the biopsy tube immediately after receiving the biopsy kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>in the refrigerator at 4°C - 8°C</td>
</tr>
</tbody>
</table>

Please store the Monovettes together with the biopsy tube, as the batch numbers must be identical.

1.3 Storing the Medicool Pack

<table>
<thead>
<tr>
<th>Storage of the Medicool Pack immediately after receiving the biopsy kit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Summer</strong></td>
</tr>
<tr>
<td>April to September</td>
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<tr>
<td><strong>Winter</strong></td>
</tr>
<tr>
<td>October to March</td>
</tr>
</tbody>
</table>

Please never freeze Medicool-Packs!

Biopsy tubes and blood Monovettes must not be used after the expiry date stated on the pack. Please check the use-by date of the biopsy kit before taking the biopsy (see label on the biopsy tube).

1.4 Informing the patient

An informative discussion is held initially with the patient, in the course of which reference is made to a possible cartilage cell removal within the framework of an arthroscopy and donation of the patient’s own blood necessary for cultivation of the cartilage cells. The “Patient information and declaration of consent” form is available for this purpose. Furthermore a form is provided for the patient, in which s/he must agree to the use of personal data and the carrying out of specific infection checks for production purposes.

Please inform your patients that in the event of positive evidence of infection (HIV, Hepatitis B, Hepatitis C, or Syphilis), Bio Tissues Technology GmbH will have to refuse to produce BioSeed®-C.

2. Taking the biopsy – blood donation

BioSeed®-C is administered in two stages: 1. Taking the cartilage biopsy, 2. Transplantation of BioSeed®-C.

2.1 Taking the biopsy

The cartilage biopsy is taken during arthroscopy.

2.2 Site for taking the biopsy

The biopsy is taken from an area of the knee less subject to stress. This is usually the medial or lateral trochlea edge (Fig. 1). Biopsies can also be taken at the edge of the fossa intercondylaris.

![Fig. 1: Lateral trochlea edge after taking 4 tissue samples with pincers.](image)

The cartilage is taken either with pincers or with a cutter. Never take the debrided tissue in the defect area as cartilage sample.

2.3 Quality of the biopsy specimen

A good biopsy can consist of one cohesive piece of tissue or 2-4 pieces (preferably the size of a grain of rice), which should weigh approx. 200-300 mg altogether.
2.4 Sending the biopsy specimens
The cartilage biopsy specimen is placed in the supplied sterile biopsy tube which contains the transport fluid. The pieces of cartilage must not come into contact with the outside or edge of the biopsy tube. The tube is then placed in the larger tube.

2.5 Blood donation
The number of blood Monovettes to be filled depends on the number of BioSeed®C grafts being produced, and on the specific country. Please consult the following table.

<table>
<thead>
<tr>
<th>Units of BioSeed®-C</th>
<th>Required blood Monovettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>Other countries</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

The blood donation can take place during the biopsy procedure. Immediately after taking the blood donation, please shake the Monovettes well so that the gel can distribute properly, then place them uncentrifuged together with the biopsy tube and the Medicool-Pack in the insulating container.

Please order more Monovettes as required.

In the last 12 hours before taking the blood donation, the patient should eat only carbohydrate-rich food which is low in fat and drink plenty of liquid. High-fat food can produce lipemic serum which is detrimental to cell proliferation. The doctor checks whether the patient is suitable for autologous blood donation.

3. After taking the biopsy

3.1 Labelling
The biopsy tube is supplied with 6 patient identification labels.

- Label No. 1/6 is for the biopsy tube. Please enter the patient’s name and date of birth, together with the sampling date and time.

- Labels No. 2/6 to 5/6 are for the production order. Please adhere one label to the original and one each to the three copies.

- Label No. 6/6 is for your patient’s medical records.

- Labels are already affixed to the blood Monovettes, bearing the same batch name as the biopsy tube.
Please mark the labels with the patient's initials to guarantee clear identification of the patient.

3.2 Post-biopsy logistics
- Place the biopsy tube with the cartilage cells and the Monovettes with the autologous blood in the insulating container together with the Medicool Pack which has been brought to the required temperature.

- Place the insulating container in the insulating box together with the fully completed, signed and labelled sampling report and production order as well as the patient consent form signed by the patient concerning the purpose, blood serum test and the use of personal data.

- The courier service collects the biopsy kit at the agreed point in time and brings it to BioTissue Technologies under defined, documented conditions within 72 hours after taking the samples.

Please note that the biopsy must be collected on the same day that it was taken.

3.3 Appointment for the operation
Please arrange the desired transplantation date with our Customer Service Department. It is not possible to change the transplantation date any later than 8 days before the agreed date. Similarly at this point cryopreservation storage is no longer possible. Please note that an additional 2 – 3 days for transport have to be added to the pure cultivation period (21 days).

4. Production of BioSeed®-C

4.1 Serology test
(HIV, Hepatitis B, Hepatitis C, Syphilis)
If the test results are negative, then cell cultivation and cryopreservation begin.

4.2 Cell cultivation
The chondrocytes are extracted from the cartilage explant by an enzymatic method and then expanded in cell cultivation bottles under GMP conditions.

4.3 Cell proliferation/expansion
Within approximately 21 days (+/- 3 days), the cartilage cells are expanded so that the optimum number of 28.8 million chondrocytes per matrix is available.

4.4 Inoculation of the support matrix
Three-dimensional distribution of the chondrocytes on the matrix.

5. Application of BioSeed®-C

5.1. Stability and Storage
BioSeed®-C can be stored after receipt until the transplantation date in the sterile packaging in the insulating container. Stability is guaranteed for 96 hours after production at a temperature of 4 – 25 ºC. After the expiry date has been exceeded, BioSeed®-C may no longer be used. Please check the expiry date (see label on insulating container or label on the end product).

5.2 Removing the defective tissue
Immediately before transplantation the whole defective cartilage tissue is removed, without causing damage to the bone lamella.

5.3 Preparing BioSeed®-C
The prepared area is measured and BioSeed®-C is cut to the corresponding size. The graft can be held in the hands or with forceps and cut to size. It is essential to avoid the graft drying out when doing this. The graft should be immersed briefly in the transport fluid or in sodium chloride solution at regular intervals. Drying out means the death of the cells!

5.4 Fixing the graft
The graft can be fixed as follows:
- Suturing the graft
- Transosseous fixing (see below)
- Resorbable pins (e.g. SmartNails®, Conmed Linvatec)
- Fibrin adhesive (for smaller defects)

Transosseous fixing
The graft is provided with a double knot loop at the corners (see Fig. 4). The thread should be at least 2.0 (Vicryl®) (surgical suture material).
Knotting technique (see Fig. 4)

<table>
<thead>
<tr>
<th>1. Diagonal stitch</th>
<th>The graft is provided with resorbable thread (Vicryl 2.0) at each corner by making a diagonal stitch.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Anchoring knot</td>
<td>At a distance of approx. 1 cm from the graft (distance depending on the site being treated), a first loop is made with a knot. The distance of the knot to the graft (and therefore the size of this first loop) is established using a needle holder. This also prevents the knot from being drawn to the edge of the graft. This anchoring knot consists of three surgical knots (4 times to the right, 4 times to the left, 4 times to the right) so that the size of the knot corresponds therefore to the diameter of the Kirschner threads.</td>
</tr>
<tr>
<td>3. Pulley loop</td>
<td>At a distance of approx. 1 cm from the first knot, a pulley loop is formed by making a second knot. This knot consists of two surgical knots (3 times to the right, three times to the left).</td>
</tr>
</tbody>
</table>

5.3 Drilling and anchoring:
The graft is fixed by anchoring knots pulled through transosseous holes drilled at the corners, with the assistance of the Kirschner wires and pulley threads (using Kirschner wires 1.7 mm thick, 35 cm long with eyelet) (see Fig. 5).

NB: Hole always drilled ventrally to the biceps tendon.

When pulling the Kirschner wires with the pulley threads, care must be taken, e.g. by providing support, to avoid strong tension directly on the graft.

Fig. 4: BioSeed®-C secured with double knot loops at the corners (with the kind consent of PD Dr. med. Ch. Erggelet, patent pending).

Fig. 5: Diagram to show transosseous 4-point fixing of BioSeed®-C in the defective area (with kind consent of PD Dr. med. Ch. Erggelet, patent pending).

The wound is closed using normal procedures.
6. Follow-up treatment of the patient

**Weeks 1 to 6**
*(Therapy with a motorised splint + partial loading)*

After the day of the operation start with passive movement exercises by means of the motorised splint, stimulating the transplanted chondrocytes to assume their natural function. The cartilage cells are thereby stimulated to form the cartilage matrix.

- Mobilisation and partial loading with 15% of the body weight (i.e. contact with the sole of the foot)
- Isometric stretching exercises

**Weeks 7 to 12**
*(Movement training)*

Gradually increase the load, special build-up training. Relieve the operated knee by means of crutches.

- Possibly ergometer training on an easy level
- Active physiotherapy

**From week 13 onwards**
*(Initial gentle sports activities):*

Gradually increase the weight load and bring muscle and coordination training up to full load condition.

- Activities with low exertion levels (e.g. cycling, jogging) as from the 6th month
- Activities with high exertion levels (e.g. tennis, football) as from the 12th month, as final maturing and hardening of the newly formed cartilage is only completed after 11 to 24 months.

**Important:** This follow-up treatment is in no way binding and acts only as a guide. The doctor carrying out the treatment will issue a detailed plan of treatment specifically for each patient.

**Pharmaceutical forms**
The following pharmaceutical forms are available:

- BioSeed®-C, measuring 30 x 20 x 2 mm (L x W x H), consists of 1 support matrix with 28.8 million autologous chondrocytes fixed with fibrin adhesive.
- BioSeed®-C, measuring 30 x 30 x 1.1 mm, consists of 1 support matrix with 28.8 million autologous chondrocytes fixed with fibrin adhesive.
- BioSeed®-C, measuring 30 x 20 x 1.1 mm, consists of 1 support matrix with 28.8 million autologous chondrocytes fixed with fibrin adhesive.
- BioSeed®-C, measuring 50 x 20 x 1.1 mm, consists of 1 support matrix with 28.8 million autologous chondrocytes fixed with fibrin adhesive.

**Licensing**
Production of BioSeed®-C requires a manufacturing permit and is carried out according to the German Drug Laws (AMG, AMWHV) and EU-GMP.

**Mandatory prescription/sales channel**
BioSeed®-C is prescribed by the doctor and delivered directly to hospitals and doctors.

**Name and address of the pharmaceutical company**
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