Technical Note

Knee Osteochondral Defect Reconstruction With Autologous Bone Grafting and Mesenchymal Cell Transplantation

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Abstract: Osteochondral defects of the knee are common in orthopaedic patients. They are challenging to treat, especially in young, highly demanding patients who do not qualify for arthroplasty. Among the many possibilities to treat osteochondral lesions presented so far, none is ideal. Because of the poor healing potential of cartilage, treatment outcomes significantly worsen with larger lesions. The treatment of large defects usually requires expensive solutions, sometimes including second-stage surgery. Using mesenchymal stem cell transplantation and cancellous bone autografts, the technique presented here for osteochondral lesion reconstruction can be effectively used to treat large osteochondral lesions in a single-stage procedure.

Osteochondral defects of the knee joint articular surface, which are common in orthopedic practice, highly impact patients’ daily life and limit sport activities. Osteochondral lesions have always been challenging to treat independently on pathogenesis, both post-traumatic lesions and due to osteochondritis dissecans (OCD), especially in young patients with high expectations.¹ The poor healing potential of hyaline cartilage is the result of limited vascularity, interaction of chondrocytes suspended in the matrix with subchondral bone and joint fluid, and the sensitivity of cartilage cells to biochemical and physical changes in the joint.¹ ² In the last 2 decades, many procedures have been developed and implemented, including gene activation matrices, autologous chondrocyte implantation (ACI), matrix-induced ACI (MACI), microfracture,
mosaicplasty, osteotomies, stem cell–coated titanium implants, and chondroprotection using pulsed electromagnetic fields, but none of these has proven to be ideal for treating osteochondral defects, especially large and deep ones.1,3,4

We present an original surgical technique for osteochondral lesion reconstruction using autologous mesenchymal stem cells embedded on a collagen implant with a 1-time autologous cancellous bone graft. The technique enables surgical reconstruction of the joint surface even in massive osteochondral lesions, for which most treatment options usually fail.

Surgical Technique

Preparation and implantation of a suspension of autologous mesenchymal cells consists of 3 stages (Video 1).

First Stage

The first stage includes patient’s qualification for the procedure. Planning osteochondral defect reconstruction surgery should include thorough assessment of the location, extent, and depth of the lesion; the mechanical axis of the limb, and the presence of any concomitant lesions [Fig 1]. Based on magnetic resonance imaging (MRI) and orthopaedic surgeon experience, as well as the availability of materials, the optimal surgical approach and the use of additional techniques, materials, or transplants may be determined.3 A thorough interview should be conducted with the patient to exclude bone marrow diseases, with a particular focus on hematological conditions. A standard morphology test with blood smear is performed. In the absence of deviations, the procedure of stimulation of the patient can be started. As a standard, 4 days before surgery, granulocyte colony-stimulating factor (G-CSF) at a dose of 480 µg/d is administered subcutaneously (Zarzio; Sandoz, Vienna, Austria). The patient is monitored for leukocytosis and possible side effects. During this period, mesenchymal stem cells multiply in the bone marrow and are gradually excreted into the peripheral blood. The increase in leukocytosis indicates the effectiveness of stimulation, and its size is usually individually variable.

Second Stage

The second stage occurs on the day of surgery and includes obtaining a suspension of CD34+ mesenchymal stem cells from the peripheral blood of the patient.5 The concentrate is obtained by apheresis using MSC blood separators under the control of the blood bank using the therapeutic leukocyte reduction protocol (Haemonetix USA) [Fig 2]. The average volume of apheresis is 1000 to 1500 ml depending on the parameters of hematocrit, sex, height, and weight of the patient.

![Fig 2. Obtaining mesenchymal stem cells from peripheral blood, using an MSC blood separator according to the therapeutic leukocyte reduction protocol (Haemonetix USA).](image)

![Fig 3. Mesenchymal stem cell suspension in a sterile bag, ready to be administered.](image)
patient. The acquisition of cellular material is carried out in accordance with the manufacturer’s instructions. With the closed-circuit technique, a suspension is obtained ready for surgical implantation [Fig 3]. A cytometric test is performed for the quantitative evaluation of cell lines as well as a histopathological evaluation of stained preparations. Cytometric evaluation of CD34\(^+\) cell lineage quantity before and after apheresis and in the resulting suspension is

Fig 4. Right knee: Patient is positioned supine. Removal of the bony cartilage sequestrum from medial femoral condyle (MFC), through enlarged anteromedial portal.

Fig 5. Using a bone spoon, the bony edges of the cavity of medial femoral condyle (MFC) are resected to the limit of healthy cartilaginous tissue (right knee, patient positioned supine, surgical access through enlarged anteromedial portal).

Fig 6. Decortication and debridement of the bottom of the medial femoral condyle (MFC) lesion using a bur. To ensure integration of bone autografts, it is necessary to remove the sclerotic subchondral bone layer until bleeding from the bone occurs (right knee, patient positioned supine, surgical access through enlarged anteromedial portal).
performed with a flow cytometer (FASCalibur, BD, San Jose, CA) using platelet sets (BD stem cell enumeration kit). Before transferring the suspension to the operating theater, compliance is ensured by checking the collection bag and the transplant metric and updating the perisurgical documentation. The cell suspension is transported in a thermobox, at ambient temperature, to an operating block, where it is transferred to the team in the preparation room.

**Third Stage**

Under local or general anesthesia, the patient is placed supine on the operating table, ensuring unrestricted access to the iliac crest on the side of the operated knee. A nonsterile thigh tourniquet is placed at the operated limb with the possibility of controlling the pressure and time of ischemia. The surgical approach is determined by the location of the lesion. When the osteochondral lesion is exposed, the delaminated cartilage or bony-cartilage sequestrum is removed [Fig 4]. Using a bone spoon, the edges of the lesion are resected to the border of healthy cartilaginous tissue [Fig 5]. The next step is decortication and debridement of the bottom [Fig 6]. To ensure the integration of bone grafts, it is necessary to remove sclerotic bone until bleeding from the bone is seen. An additional factor improving the blood supply is microfracturing the bottom of the cavity.

From a separate cut above the iliac crest, autologous cancellous bone grafts are harvested [Fig 7]. To reduce operating time, graft harvesting should be carried out simultaneously by a second operating team. To achieve
In the early postoperative period, it is recommended to elevate the operated limb to reduce swelling. In the process of postoperative rehabilitation, the authors postulate partial weightbearing of the operated limb for 9 weeks. During this time, continuous passive motion machine is advised in the patient’s accepted range of motion (ROM) for 5 to 6 hours a day. Specific rehabilitation protocols usually depend on possible concomitant injuries.\cite{6}

**Discussion**

The presented surgical technique can be used for reconstruction of osteochondral defects regardless of their size, while most other treatment options require intact subchondral bone, and their healing efficiency decreases with increasing size of the lesion\cite{3} [Fig 12]. As a result of G-CSF stimulation, young mesenchymal cells of CD34\(^+\) lineage multiply and are a source of growth factors as well as cell lines in further proliferation and remodeling processes.\cite{7,8} This allows the procedure to be completed in a single surgery, reducing stress on the patient and removing the risk of a second surgery for autologous chondrocyte implantation. The amount of CD34\(^+\) cells obtained by the apheresis process reaches on average 2 to 3 \(\times 10^6\) in 1-cm\(^3\) suspension, which is confirmed each time using cytometry. The cell separation technique also means that the minimum necessary volume of cell concentrate needed for reconstruction can be planned.\cite{5} Autologous mesenchymal stem cells, unlike chondrocyte culture suspensions, are resistant to physicochemical changes occurring at the site of

![Fig 11](https://example.com/fig11.png)

**Fig 11.** The osteochondral reconstruction is covered with a 2-component fibrin glue. After binding, a suspension of mesenchymal cells is administered under the fibrin layer using a no. 12 needle, and the application site is sealed with the rest of the glue (right knee, medial femoral condyle, patient positioned supine, surgical access through enlarged anteromedial portal).

![Fig 12](https://example.com/fig12.png)

**Fig 12.** Postoperative magnetic resonance imaging performed 2 years after surgery reveals reconstructed lesion (arrow) of the medial femoral condyle (MFC) joint surface (right knee, sagittal scan).
reconstruction, which facilitates both the course of surgery and rehabilitation of the patient.7

Another advantage of this procedure is the significantly lower cost of obtaining a cell suspension. Cells are obtained from peripheral blood after G-CSF administration, which causes stem cells to excrete from bone marrow to bloodstream. This feature can also be used in cases of lesions small enough to not require bone graft transplantation, resulting in less trauma.8-11

Autologous cancellous bone grafts have an advantage over bone replacement material in bone integration during healing, facilitating faster recovery, and are preferred when performing the reconstruction procedure. Arthroscopy performed before addressing the osteochondral lesion allows treatment of any concomitant injuries in the knee and confirms mini-open access localization. The mini-open technique facilitates visualization of the lesion and tool maneuvering inside the knee, therefore reducing time of procedure and allowing better bed preparation for transplantation. Access to lesions varies and is determined by the location of the osteochondral defect. If possible, a skin cut should be performed through an existing arthroscopic portal to minimize soft tissue damage.

As in every surgical procedure, there are some risks and disadvantages. The most important are donor site morbidity due to cancellous bone autograft harvesting and possible side effects of using G-CSF [Table 1]. On the first postoperative day, significant joint edema can be observed. Knee joint aspiration is not indicated because of the risk of affecting the reconstruction. The edema resolves spontaneously, usually in a week.

Osteochondral lesion reconstruction, with the support of a single bone lesion reconstruction using autologous cancellous bone graft and a suspension of autologous mesenchymal stem cells embedded on the collagen membrane, is a valuable method and can be an alternative to previously reported methods. Despite promising results in subjective assessment, objective assessment of long-term results has yet to be done. It is worth noting that large lesions, which usually prove to be the most difficult to treat, can be addressed, because the cell transplantation techniques produce more cellular material than other methods, creating a stable basis for cartilage reconstruction.

Table 1. Advantages and disadvantages

| Advantages | Disadvantages |
|------------|--------------|
| Lesions of every size can be addressed | Possible donor site morbidity |
| Large amount of MSCs can be obtained | Risk of G-CSF side effects |
| Cancellous bone autograft has better healing properties than bone substitute materials | Lack of long-term data |
| One-stage surgical procedure | Requires longer preparation for MSC stimulation |
| Blood-harvested MSCs have increased resistance to physiochemical changes, compared with chondrocyte cultures | Mini-open technique causes more tissue damage |
| Mini-open technique facilitates better access to lesion | Significant joint swelling in early postoperative period |
| Mini-open technique facilitates tool maneuvering inside the knee | |

Abbreviations: G-CSF, granulocyte colony-stimulating factor; MSC, mesenchymal stem cell.

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