Histopathological examination in evaluation of long-term results after osteocartilaginous transplantation

R. RĂDULESCU1,2), A. BĂDĂILĂ2), O. NUŢIU2), R. MANOLESCU2), SILVIA TERINTE2), MARIA SAJIN1,3), I. M. JAPIE2)

1) "Carol Davila" University of Medicine and Pharmacy, Bucharest
2) Department of Orthopedic Surgery
3) Department of Pathology
University Emergency Hospital, Bucharest

Abstract
Objective: To assess long-term outcomes of osteocartilaginous transplantation for non-degenerative lesions of hyaline articular cartilage in the knee, by performing minibiopsies from the transplanted area and examining them histopathologically. Patients and Methods: Forty-four patients with post-traumatic cartilage injuries of the bearing surfaces of the knee were enrolled in a prospective study, that included treatment with autologous osteocartilaginous grafts at the level of the lesion, “second look” arthroscopy and targeted minibiopsies at one year and five years postoperatively (six minibiopsies per patient). The collected tissue fragments were examined by optic microscopy. In order to integrate the histopathological findings in the clinical context, the function of the knee was also quantified by calculation of the International Cartilage Repair Society Score preoperatively, at one year and at five years postoperatively. Results: Five years post-transplant the outcomes for 36 patients were available. One year post-transplant, the histopathological examination revealed the presence of hyaline cartilage in 165 of the 216 (76.39%) tissue samples collected and fibrocartilage in 51 (23.61%) respectively. Five years after surgery, the proportions of these findings were 159/216 (73.61%) for hyaline cartilage and 57/216 (26.39%) for fibrocartilage. The difference was not statistically significant (p>0.1). The evolution of the ICRS clinical score was from 38.57±3.42 preoperatively to 80.31±3.85 (p<0.0001) after one year and to 81.35±4.57, respectively at five years after surgery. Conclusions: Autologous osteocartilaginous transplantation brings hyaline articular cartilage at the level of the injured area. Approximately three quarters of the surface lesion remains covered by high quality hyaline cartilage that maintains its macroscopic structure and architecture for a long period of time.

Keywords: autotransplant, hyaline cartilage, biopsy, histopathology.

Introduction
The articular cartilage is an avascular and aneural tissue. The lack of both blood circulation and lymphatics can explain, at least partially, the very low intrinsic ability of self-repair. Cartilage injury does not result in local inflammatory response and, as such, there is no macrophage invasion, which through phagocytosis would remove the damaged and devitalized tissue. Also, there is no local cellular activation, no local cellular multiplication and no migration of different cells with reparative capacity. Chondrocytes are isolated in their lacunae and so they cannot migrate to repopulate the injured area. Therefore, chondral lesions rarely heal themselves (except those with less than 0.5 cm in diameter).

In order to prevent the development of osteoarthritic injuries, in non-degenerative cartilage injuries larger than 0.5 cm in diameter, the cartilage should be replaced with hyaline cartilage tissue with a high mechanical resistance. This will guarantee the endurance in time despite prolonged cyclical mechanical stress.

The only therapeutic method that contributes to the healing of structural hyaline articular cartilage at the level of the injured area, with the scope of absorbing the mechanical stresses, is autologous osteocartilaginous transplantation from the non-bearing areas of the joint to the site of lesion, which is usually located on the bearing area.

Transplantation implies harvesting areas of osteocartilaginous grafts at the level of the lesion, “second look” arthroscopy and targeted minibiopsies at one year and five years postoperatively (six minibiopsies per patient). The collected tissue fragments were examined by optic microscopy. In order to integrate the histopathological findings in the clinical context, the function of the knee was also quantified by calculation of the International Cartilage Repair Society Score preoperatively, at one year and at five years postoperatively. Results: Five years post-transplant the outcomes for 36 patients were available. One year post-transplant, the histopathological examination revealed the presence of hyaline cartilage in 165 of the 216 (76.39%) tissue samples collected and fibrocartilage in 51 (23.61%) respectively. Five years after surgery, the proportions of these findings were 159/216 (73.61%) for hyaline cartilage and 57/216 (26.39%) for fibrocartilage. The difference was not statistically significant (p>0.1). The evolution of the ICRS clinical score was from 38.57±3.42 preoperatively to 80.31±3.85 (p<0.0001) after one year and to 81.35±4.57, respectively at five years after surgery. Conclusions: Autologous osteocartilaginous transplantation brings hyaline articular cartilage at the level of the injured area. Approximately three quarters of the surface lesion remains covered by high quality hyaline cartilage that maintains its macroscopic structure and architecture for a long period of time.

Keywords: autotransplant, hyaline cartilage, biopsy, histopathology.
Orthopedics and Traumatology at the University Emergency Hospital of Bucharest, Romania, and initially included a total of 44 patients. It was a prospective study, extended over a period of 10 years (2002–2011). Gender distribution of the initial batch was 29 men/15 women. At the final review of the results, complete data were available for 36 cases (sex ratio: 24 men/12 women). The average age for the final batch was 27 years and 8 months ± 5 years and 4 months with a range of 18 to 58 years.

Inclusion criteria were:

- The existence of a type III or IV Outerbridge [1] cartilage or osteocartilaginous nondegenerative injury of the knee;
- Limited lesion, with an area less than 6 cm²;
- Lesion located in the bearing areas;
- High quality, non-bearing, donor area cartilage;
- The absence of degenerative pathology, except for the chondral lesion;
- The absence of any ligament or inflammatory pathology located at the knee;
- Normal axial alignment of the knee;
- Patient compliant to the requirement of walking without weight bearing for intervals of 6 weeks or more;
- Patient able to follow the indicated physical rehabilitation;
- Good general health and cognitive capacity of the patient in order to make him eligible for such surgery and able to fill average complexity forms;
- Signed patient informed consent to be included in the study and to submit its rigors.

According to the Outerbridge [1] classification, 11 lesions were type III and 33 were type IV.

Preoperatively the ICRS [2, 3] score was determined for each patient (International Cartilage Repair Society).

The surgical treatment [4–6] consisted of:

1°. Injured area preparation: removal of damaged cartilage and scar tissue and designing drilling tunnels through the bone, in order to obtain grafts to cover the whole surface of the injury, maintaining bone walls of at least 1 mm thickness between the tunnels.

2°. Collecting osteocartilaginous drill core from donor areas (femoral trochlea edges outside the articular surface corresponding to the patella and the edges of the intercondylar fossa).

3°. Planning of graft insertions according to their length and especially to the shape of the articular surface, which is to be reconstructed and to the orientation of the cartilage surface to the long axis of the drill cores. The goal is to orient the grafts for them to be as congruent as possible to the adjacent cartilage surface and the general borders of the area.

4°. Inserting the osteocartilaginous cylindrical grafts into the tunnels drilled at the level of the lesion, in order to obtain as smooth a joint surface as possible, best integrated into the overall architecture of the area (curves, levels, borders) (Figure 1).

Figure 1 – Intraoperative picture: osteocartilaginous lesion of the medial femoral condyle prepared for autografting (left) and after insertion of the osteocartilaginous cylindrical grafts (right).

One year after surgery, the clinical score was recalculated and six minibiopsies (Figure 2) were collected (three from the central and three from the peripheral area) from the surgically repaired area using the arthroscopic approach.

On the same occasion, macroscopic arthroscopic inspection (Figure 3) of the autotransplantation area was performed in order to assess its articular surface, color, texture and its congruence with the normal adjacent cartilage.

The small fragments collected were examined by optical microscopy, processed in paraffin and stained with Hematoxylin–Eosin and Van Gieson. We observed the existing type of cartilage, its structure, viability and cellularity.

Five years after surgery, we performed the same steps: recalculating the clinical score, collecting the minibiopsies from the former lesions, processing the collected small fragments and determining the existing cartilage type, structure, viability and cellularity. Five years after surgery, the results were available for 36 patients.
Histopathological examination in evaluation of long-term results after osteocartilaginous transplantation

Results

One year after surgery, the histopathological examination revealed the presence of hyaline cartilage (Figure 4) in 165 (76.39%) and fibrocartilage (Figure 5) in 51 (23.61%) from the 216 collected cartilage fragments.

Three (8.33%) cases have showed moderate hypocellularity of the transplanted hyaline cartilage (defined as a proportion of lacunae without containing viable chondrocytes between 20% and 50% of all lacunae visualized on the microscope field), and two (5.55%) cases showed marked hypocellularity (defined as a proportion of lacunae without containing viable chondrocytes greater than 50% of all lacunae visualized). Hyaline cartilage maintained viability and microarchitecture in all cases.

On the long term, five years after surgery, the proportions were 159/216 (73.61%) for hyaline cartilage (Figure 6) presence and 57/216 (26.39%) for fibrocartilage (Figure 7) presence. The difference is not statistically significant ($p>0.1$).

Five years after surgery, moderate hypocellularity was observed in four (11.11%) cases and marked hypocellularity in two cases (5.55% – the same two cases from the first year check-up postoperatively). These two latter cases have shown degradation of tissue structure. At the macroscopic inspection performed arthroscopically, we found that the injured surface, which was repaired surgically, took a fibrillar pattern. Clinically, in these two cases the results were mediocre – at one year postoperative, the ICRS scores were of 68 in one case and 70 in the other, and five years after surgery – 66 and 69 points respectively.

The evolution of the ICRS clinical score was from the preoperative value of 38.57±3.42 points (mean value ± standard deviation) to 80.31±3.85 points one year postoperatively and 81.35±4.57 points five years postoperatively.
Discussion

Osteochondral lesions are very common; in almost half of the surgical interventions performed arthroscopically, chondral lesions are identified; they are often asymptomatic (arthroscopy is usually performed for other injuries: meniscal or ligamentary). Many injuries are diagnosed in young patients. The goals of the treatment are to obtain a functional joint (quasinormal mobility, reasonable exercise capacity, strength, etc.) without associated symptoms (pain, laxity, locking, swelling, etc.) and to prevent the development of degenerative lesions. These goals are not temporally punctual, but they must prove duration over several decades of life.

Optimal healing of a chondral or osteochondral lesion occurs when it becomes covered with tissue of similar mechanical strength as the hyaline cartilage, and integrates well into the normal adjacent cartilage, restoring congruence with the adjacent and contralateral articular surface. The graft’s mechanical resistance has to be constant over time and ideally integrate progressively into the normal turnover of the articular cartilage.

Multiple surgical techniques are proposed to achieve these objectives: abrasion arthroplasty, drillings, microfractures, periosteum or pericondrium transplantation, autologous osteochondral graft transplantation or osteochondral allografts, autologous chondrocyte transplantation, etc each with its variants. They range in difficulty from simple (drillings or microfractures) to complex (osteochondral graft transplantation or autologous chondrocyte transplantation). There are two concepts of treatment for non-degenerative cartilage lesions: the first is based on creating a communication between the injured area and the subchondral cancellous bone by which the multipotent mesenchymal cells existing at this level would migrate and differentiate progressively on the chondrocyte line, while the second concept is based on the principle of collecting hyaline cartilage from a healthy area and transplanting it at the level of the lesion.

Along with the subchondral bone, the cartilage forms a morphofunctional unit (Figure 8).

Based on such a finding, widely accepted by many authors, different authors reach different conclusions: if supporters of autologous osteocartilaginous transfer state that this technique has the advantage of enhancing the number of hyaline cartilage-subchondral bone units at the level of the lesion, the proponents of autologous chondrocyte transplantation argue that one of the strong points of their method is indeed that the subchondral bone is left as it is and that the methods involving penetration of subchondral bone lead to its hardening and changing of the pattern of mechanical solicitations involving the cartilage.

All these surgical techniques are based on biological...
principles, all are based on studies that support their efficacy and have their supporters and their critics. The fact that there are so many proposed techniques, that their application is simultaneous and that their indications overlap show that neither of them meets the desired consensus and that none of them has succeeded in establishing itself as the best way to resolve osteochondral lesions. Therefore, we considered that this segment of modern orthopedic pathology and therapeutics offers a wide horizon for research, as current techniques can still be improved, compared and associated with their optimal indications.

Hangody L et al. was the first to imagine the multiple osteocartilaginous autografts transplantation [7–9] this also being the subject of his doctoral thesis held in 1994 at the Semmelweis University in Budapest, Hungary. After a period of experiments between 1990 and 1991 carried on an animal model, on February 6, 1992, he proceeded with the first intervention of this kind on a human patient. He published his results in 1994 and although he was surpassed by Matsusue Y et al. [10], who described a similar procedure in 1993, in Arthroscopy, he is still recognized as the first one to conduct experiments and interventions of such type.

Since the late 90’s, this technique was also practiced in our country, as University Emergency Hospital of Bucharest one of the first centers to perform it. Our hospital now has one of the most extensive and complex experiences with this procedure and also one of the richest casuistry. The current study aims to determine, at a macroscopic level, the evolution in time of the transplanted hyaline cartilage. This is only possible through a minimally invasive maneuver – collecting mini-biopsies under arthroscopic control. Informed consent was obtained from patients at the time of enrollment in the study. To avoid compromising articular cartilage, the biopsy collection was performed by needle-biopsy [2, 3] technique.

Examination by optic microscopy revealed a surface coverage rate with hyaline articular cartilage of about three quarters. The spaces between osteocartilaginous transplanted cylindrical autografts were filled with fibrocartilage. Both types of cartilage are connective tissues consisting of cartilage cells (chondrocytes) and extracellular matrix (fundamental substance and connective fibers). There are several important differences between them. The hyaline cartilage has a more evident cellularity – chondrocytes that occupy lacunae in the fundamental substance, while the fibrocartilage is richer in collagen fibers. Chondrocytes from the hyaline cartilaginous tissue may appear aggregated in isogenic groups (they appear by cell mitosis – the process by which cartilage develops in the interstitium). Interstitial growth of cartilage occurs in adults too, but is far more limited, while at the embryo and fetus this is one of the main growth processes.

In the hyaline cartilage, the superficial lacunae are filled with a single chondrocyte, while those located deeper contain multiple chondrocytes. Here, chondrocytes are surrounded by a richer in glycosaminoglycans fundamental substance than the rest of the cartilage – territorial matrix, which, stained with Hematoxylin–Eosin, appears basophilic at microscopy. The remaining fundamental substance consists of extraterritorial matrix, which is less basophilic and paler when stained with Hematoxylin–Eosin. Van Gieson stain method (Picric Acid and Fuchsins Acid) (Figure 9) stains collagen fibers in shades ranging from pink to dark red. Because of this, the territorial matrix will appear paler and the extraterritorial matrix will look more intense colored, in red shades.

Figure 9 – Hyaline cartilage and bone–cartilage junction (Van Gieson staining, 400×).

The hyaline cartilage essentially consists of water (65–80%), collagen type II (10–20%) and proteoglycans. Other minor components are the proteins, lipids and phospholipids, and other types of collagen in small quantities. Collagen has a triple helix structure and the alignment of the fibers and intermolecular connections lead to the formation of fibrils. Aggrecan is the main proteoglycan present in cartilage structures – composed by a central protein that attaches lateral chains of chondroitin sulfate and keratan sulfate. Aggrecan molecules are attached by a link protein to the hyalurionate molecule, forming a macromolecular complex.

Extracellular matrix can be described as a biphasic structure – a solid phase consisting of collagen and proteoglycans and a fluid phase consisting of water and ions. The solid phase has a low permeability to fluids due to its high frictional resistance. Water is maintained in the interfibrillar spaces by the negative charge of the proteoglycans. All these make the fluid phase suffer a high pressurization, thus the cartilage becoming viscoelastic and able to take-up large and repeated cyclic mechanical stress without any injury.

All other tissues fail to present the mechanical performance of a healthy cartilage, thus underlining the importance of enhancing the quality and quantity of hyaline cartilage at the level of articular bearing surfaces. This is achieved by the osteocartilaginous autologous transplantation, which provides integration of transplanted tissues through osteointegration and revascularization of the bony component of the drill graft core.

The histopathology examination performed at one year and five years post-transplant showed that in most cases the transplanted hyaline cartilage maintained its structure, cellularity and viability. Hypocellularity was observed in 13.88% of cases at one year post-transplant.
R. Rădulescu et al.

(marked hypocellularity in 5.55% of cases) and in 16.66% at five years postoperatively, (marked in 5.55% of cases).

We can conclude, on one hand, that if the transplanted cartilage cellularity is damaged, this happens during the first year, and, on the other hand, that if this first critical year is successfully surpassed, the microscopic results (cartilage viability) will last for at least five years. There is a comment to be made, in order to objectively evaluate these results, tissue fragments from drill cores should have been collected at the zero moment of implantation to see whether the hypocellularity pre-existed.

The reduced cell load was associated with a degradation of cartilage in time, namely changes in the microscopic structure and a fibrillar pattern of the articular surface at an arthroscopic inspection. This hypocellularity was associated with modest clinical outcomes – ICRS scores at the lower level of the spectrum.

In clinical terms, autologous osteocartilaginous transplantation had very good results, as improvement of symptoms at one year after surgery showed high statistical significance \(p<0.0001\). Clinical results are maintained over time, clinical score values showing minimum variation between the first and the fifth year postoperatively, without statistical significance \(p>0.05\).

\section*{Conclusions}

Osteocartilaginous autologous transplantation enhances the quality and quantity of the viable hyaline cartilage at the injured area. Approximately three quarters of the lesion area is thus covered with high quality hyaline cartilage that keeps its structure and macroscopic architecture on a long term. Hypocellularity of the area treated with osteocartilaginous autologous transplantation is associated with poorer clinical outcome. Clinically, the joint function was improved (statistically significant), the results lasting for at least five years postoperatively.

\section*{References}


\section*{Corresponding author}
Adrian Bădilă, MD, PhD, Department of Orthopedics and Traumatology, University Emergency Hospital Bucharest, 169 Independenţei Avenue, Sector 5, 050098 Bucharest, Romania; Phone: +40722–516 470, e-mail: adrian_emilian_badila@yahoo.com

Received: April 12, 2013

Accepted: September 2, 2013