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Arthroscopic second generation autologous chondrocyte implantation at 48 months follow-up

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Autologous Chondrocyte Implantation (ACI) proposed by Brittberg in 1994 [1], involving re-implantation of autologous cells that have been isolated from cartilage harvested from the patient and expanded in vitro, is gaining wide scientific and clinical support for use in the repair of focal articular cartilage lesions. A second-generation tissue-engineering approach to cartilage repair was proposed, which uses a biodegradable three-dimensional scaffold for cell proliferation. This scaffold is entirely based on the benzylic ester of hyaluronic acid (HYAFF® 11, Fidia Advanced Biopolymers Laboratories, Padova, Italy) and consists of a network of 20-µm-thick fibers with interstices of variable sizes, and has been demonstrated to be an optimal physical support to allow cell-cell contacts, cluster formation, and extracellular matrix deposition [2,3,4]. The cells harvested from the patient are expanded and then seeded onto the scaffold to create the tissue-engineered product Hyalograft C. Seeded on the scaffold the cells are able to re-differentiate and retain a chondrocytic phenotype even after a long period of in vitro expansion in monolayer culture [5,6,7,8]. The efficacy of the cell-scaffold construct was also proven by in vivo implantation in an animal model [3]. Hyalograft C constructs can be implanted by press-fitting directly into the lesion, thus avoiding suturing to surrounding cartilage and obviating the need for a periosteal flap [9,10]. Moreover, the features of this device have permitted the development of an arthroscopic surgical technique for implantation of autologous chondrocytes on a hyaluronic acid support with the aim of reducing patient morbidity, surgical time and recovery and complications related to open surgery [9]. The results of Hyalograft C implantation under open procedure without the use of a periosteal flap are encouraging [11, 12]. Histological analysis of biopsies obtained from the patients who had undergone autologous chondrocyte culture and Hyalograft C application without a periosteal coverage, have shown a hyaline-like cartilage in the implant zone just 12 months after the implant [10,11, 13]. The purpose of this study is to study the possibility of treating femoral condyle chondral lesions with arthroscopic implantation of Hyalograft C and analyze the indication criteria for this type of treatment. For this purpose a group of 54 consecutive patients treated by the arthroscopic technique was prospectively evaluated at a minimum of four years follow-up.

Patient selection. Arthroscopic techniques have been developed and used in our Institute since 2001. Clinical experimentation was approved by the Hospital Ethics Committee and informed consent of all patients was obtained. The treatment was indicated in patients with focal chondral defects involving femoral condyles and trochlea, complaining of clinical symptoms (pain, swelling, locking, and giving way). Exclusion criteria were age <15 and >60 years, untreated tibio-femoral or patello-femoral malalignment or instability, diffused arthritis or bipolar ("kissing") lesions, and those with other general medical conditions (diabetes, rheumatoid arthritis, etc). All patients gave their consent to participate and comply with the required post-operative rehabilitation regimen and were included in the study. All the patients were consecutively treated and prospectively evaluated. 54 consecutive patients treated by arthroscopy with a minimum of 48 months follow-up were prospectively evaluated. The lesions treated were single in 51 cases and multiple in 3 cases (medial femoral condyle combined with trochlea lesions in 2 cases and medial and lateral condyle lesions in one case). 38 chondral defects were situated on the medial condyle, 15 on the lateral femoral condyle, and 4 on the trochlea. All lesions were Outerbridge grade III-IV and the mean size of the lesions was 2.4 cm2. (1.5-5 cm2). The etiology was traumatic in 27 cases, degenerative in 20 cases, and 7 patients were affected by osteochondritis dissecans. The mean age of the patients was 29 years (range 15 - 60 years). The patients practiced sport at a highly competitive level in 19 cases, at amateur level in 29 cases, while 6 patients did not practice any type of sport. 22 patients underwent previous surgery: 7 meniscectomies, 8 ACL reconstructions and 8 cartilage repair operations, such as shaving, debridement or microfracturing of chondral lesions and mosaicplasty. In 26 patients associated procedures were performed during cartilage harvesting: 18 ACL reconstructions, 17 meniscectomies, 3 meniscal sutures, 1 collagen meniscus implant (Tab.1). In OCD cases, 5 patients presented a 10-12 mm deep defect and were treated with arthroscopic autologous bone grafting during the cartilage harvesting procedure. In remaining 2 OCD cases with 3-7 mm deep bone defect no associated procedures were performed. In 21 cases the meniscus was partially removed from the same compartment of the chondral lesion during previous or associated surgery. The number of Hyalograft C patches used was between

1 and 4, of 2 different diameters (6.5 mm-8.5 mm). Chondrocyte harvesting and culturing The surgical technique for ACI consists of two steps. The first procedure consists of a biopsy of healthy cartilage for autologous chondrocyte cell culture. This is usually performed arthroscopically, when the chondral lesion has been observed, and the indication for ACI has been made. A 150-200 mg cartilage biopsy is taken from a non-weight bearing site of the articular surface (intercondylar notch) and sent to the processing center in a serum-free nutritional medium. The following day the tissue is minced into smaller pieces and digested with 0.25% trypsin at 37 °C for 15 min and then with 300 U/ml collagenase type II (Worthington, Lakewood, NJ, USA) at 37 °C for 4 hr in Ham's F12. The digested material is centrifuged at 1000 rpm for 10 min and the pellet is resuspended in Ham's F12 containing 10% fetal calf serum (Sigma), 1% penicillin-streptomycin, 1% L-glutamine, 1 ng/ml TGFβ 1, 1 ng/ml insulin, 1 ng/ml EGF, and 10 ng/ml bFGF (all growth factors were recombinant and of human sequence). Typically from 200 mg of tissue 1 − 2 million cells are recovered. Cells are amplified in monolayer cultures up to three passages, then they are seeded onto Hyalograft C scaffolds (2 x 2 cm). 8 x 106 cells are resuspended in 0.4 ml of medium (as above, but containing 50 ïg/ml ascorbic acid), the cell suspension is pipetted onto the scaffold and the culture is kept at 37 °C, 5% CO2 overnight, The next day, additional medium is added to submerge the cell construct completely, whereas the medium is changed twice a week. Hyalograft CO chondrocyte cultures are ready for shipment after 2 weeks in culture. The day of shipment the cell construct is washed exhaustively with PBS then sealed in a sterile plastic tray containing 4 ml of nutritional medium. The expiry time of the product is 72 hr. Arthroscopic surgical technique According to the technique developed [9] a variable diameter (6.5 mm − 8.5 mm) delivery device with a sharp edge is used to evaluate the size of the defect in order to ensure a complete coverage of the defect. A flipped cannula that allows the removal of the fat pad from operative field is then inserted into the antero-medial portal and a specifically designed cannulated low profile drill, (6.5 mm–8.5 mm) maintained in the selected position by a Kirschner guide wire (0.9 mm diameter), is used to debride the lesion and make a circular area with regular margins for graft implantation. The delivery device is then filled with a hyaluronic acid patch, which is transported and positioned in the prepared area. The graft is pushed out of the delivery device and precisely positioned within the defect where it remains fixed tightly to the subchondral bone. The stamps can be overlapped, but the positioning inside the prepared circular area with good shoulder healthy cartilage coverage is mandatory. Under arthroscopic control the stability of implanted stamps is evaluated also during cyclic bending of the knee. Mobilization of the implanted patch was not observed in our series (Fig. 1). Follow-up evaluation All 54 patients were clinically prospectively evaluated preoperatively and at 24 and 48 months follow-up. The 28 patients with isolated chondral lesions were evaluated separately and compared to the patients who underwent associated procedures. The International Cartilage Repair Society Evaluation Package [14] was used for clinical evaluation. Patients were also asked to evaluate their quality of life using the EQ-VAS score [15]. Returning back to sport was also recorded at 24 and 48 months follow up, evaluated with Tegner Score and compared with pre-operative and pre-injury level. At a follow-up of 12-36 months, 11 second-look arthroscopies was performed, with patient consent, for investigative purposes to check visually and probe for consistency of the implanted cartilage. Repair tissue quality was classified according to the Brittberg score[16]. During the second-look arthroscopy, full-depth biopsies of cartilage and subchondral bone were obtained from the repair site in 2 patients, who expressed their consent for a follow-up biopsy. As the grading of matrix protein abundance by histological analyses alone may be misleading [17], both histological and quantitative biochemical analyses were performed on each biopsy [18] assays and total collagen was measured as hydroxyproline by amino acid analysis [13,17]. Statistical Methods All continuous variables were expressed in terms of mean ± standard deviation of the mean. The Paired t test was performed to test the hypotheses about means at different follow up times when the data were normally distributed; otherwise the Wilcoxon Test was used. One Way ANOVA was performed to test hypotheses about means of different groups. The Mann Whitney Test was used to test the hypotheses about continuous data differences between groups or among groups. Rank Spearman Correlation was performed to investigate relationships between two quantitative measurements, For all tests p<0.05 was considered significant. Statistical analysis was performed using SPSS 7.5 (Rizzoli Orthopaedic Institute).

No complications related to the implant or serious adverse events were observed during the treatment and follow up period. A statistically significant improvement in all scores was observed after 24 months. The improvements remained stable over time at 48 months. A statistically significant improvement in the IKDC objective

score was observed (Wilcoxon Test p<0,0005), showing a normal or nearly normal knee in 83.3 % (45/54) of patients at 24 months follow up and in 85 % (46/54) of patients at 24 months follow up (Tab. 2). Worsening of IKDC objective score was observed in 2 patients at 48 months. One of these 2 patients underwent medial meniscal removal previously. There was no statistically significant difference between results at 24 and 48 months follow up. Statistical analysis shows a significant improvement in the IKDC subjective score and self-assessment of quality of life (EQ-VAS) from pre-operative to 24 and 48 months (Paired T-test; p<0.0005), with no significant difference between 24 and 48 months (Tab. 3,4). The mean IKDC score improvement at 24 months was 39±23 and 39±26 at 48 months follow up, whereas the mean improvement with the EQ-VAS score was 16±26 at 24 months and 19±22 at 48 months follow up. The mean Tegner score was 6.34 (2.2 s.d.) before the injuries, 1.72 (1.1 s.d.) before the treatment, 4.88 (2.6 s.d.) at 24 moths follow up and 4.94 (2.7 s.d.) after 48 months. These results showed a statistically significant improvement (p< 0.0005) after the surgery at 24 and 48 months follow up; however the new level of sport activity (Tegner 4.94) was significantly lower with respect to the pre-injury level (Tegner 6.34) (p<0.0005) (Tab.5). In order to establish the indications for this type of treatment, we tried to determine the parameters that influence the clinical outcome. Statistical analysis (Rank Spearman Correlation) showed better results in younger patients with subjective IKDC evaluation at 24 (p<0,001; Rho= -0,447) and 48 moths follow up (p<0,005; Rho= -0,377). Better results were also achieved in well-trained patients. Our results did not show any significant difference between previously treated and never treated patients at 48 months follow up, only at 24 moths there was a difference in clinical outcome, with better improvement of never treated patients, but not statistically significant (p=0,006 One Way Anova). The patients who underwent associated surgery had similar clinical results to patients with isolated chondral lesions. Other factors, such as defect size and localization, did not influence significantly the results. Arthroscopic evaluation revealed a complete coverage of the grafted area with a hyaline cartilage-like tissue, filling of the defect, and integration with the original, surrounding cartilage in 7 of 11 analyzed cases. In 2 cases poor integration of the new cartilage with the border zone and in 3 cases irregularity of cartilage surface was found. Mean Brittberg defect repair score was 10.5. We undertook histological evaluation of the regenerating tissue, albeit after a short follow up of 12 months. In these 2 cases there was clear evidence of regenerated cartilage that was hyaline in one case and fibrocartilage in the other [18]. Both specimens showed close integration of the engineered cartilage with the subchondral bone. Moreover, a clear presence of a tidemark, which is typical of hyaline cartilage and is rarely seen in cartilage repair biopsies, was observed particularly in one case [18].

The arthroscopic autologous chondrocyte implantation technique was developed in the attempt to improve the efficacy and reduce the morbidity of the ACI technique, which still remains for us one of the main concerns of this technique [19]. Recent prospective studies [20,21,22] have not completely clarified the better performance of the ACI technique compared to other procedures used for cartilage repair. Knutsen [22], in particular, reported similar results for microfracture and traditional ACI technique at 2 years follow up. The necessity to create a hermetic periosteum seal using sutures, the requirement of a second open surgery and possible complications related to the use of a periosteal flap can explain these results. There is a need for a large joint exposure wich increases morbidity for the patients and produces a higher risk of joint stiffness and arthrofibrosis, as frequently observed with this procedure. Periosteal hypertrophy occurs between 3 and 7 months after surgery in 10-25% of cases and often requires revision surgery [23,16]. Some authors [19] have shown a reoperation rate of up to 42%, due to joint stiffness. Some authors [19,24,21] have indicated that the use of the periosteal flap increases the risk of complications during the recovery period and produces more difficult rehabilitation. The most frequent motivation for revision surgery is the incomplete periosteal graft incorporation to the host cartilage and hypertrophic graft edge response. The use of a three-dimensional scaffold for the cell culture with open surgery already permits a reduction of joint exposure because it avoids periosteal harvesting and suturing. The development of the arthroscopic technique reduces morbidity for the patient, recovery time and rehabilitation protocol, thus facilitating recovery for the patients. Arthroscopic autologous chondrocyte transplantation maintains the advantage of the osteochondral grafting arthroscopic procedures, while the disadvantages of donor site morbidity can be avoided, particularly when we have to treat large defects. In fact, it is possible to treat more extensive lesions without problems of material availability. Moreover, patches can be overlapped to create a complete coverage of the defect without leaving uncovered interval spaces typically observed in lesions treated with osteochondral grafting. No implant related complications were noted in this study. Even in cases where more than 2 patches were used with overlapping of the grafts, no symptoms related to overgrowth or hypertrophy were observed. Satisfactory clinical results were achieved at 2 and 4 years follow-up in our series, and only patients with femoral condyle or troclear chondral lesions, treated by arthroscopic surgery, were considered to form a homogeneous group. Patients with patellar or tibial plateau lesions were excluded from the study in order to avoid bias. We noted that the results with bioengineered cartilage were better in young patients who practiced sport at a highly competitive level. This observation can be correlated to the greater commitment of this patient to the rehabilitation program, but also confirms the possible functional recovery of the knee after this procedure that meets the high functional request of competitive athletes. The patients were not permitted to return to the sport activity before 12 months in order to guarantee initial cartilage tissue stability and maturation. This fact can be a disadvantage for athletes with highly competitive requests and necessity of the quick return to sport activity. The results were not compromised by associated surgery, which suggests that this procedure may be combined successfully with different surgical operations. All the associated procedures were performed in the same surgical session as cartilage harvesting, in order to avoid surgical stress and favour the rehabilitation program of chondrocyte implantation. No statistically significant difference respect to the group with associated lesions was detected. This suggests that the clinical improvement of these patients was related to the cartilage surgery. Anyway it is very difficult to stabilize the influence of concomitant lesions on the clinical outcome and the number of the patients who underwent associated surgery represents one of the most important biases of this study. We have noticed also that our results were not affected by defect size. In fact, satisfactory results were obtained also in large cartilage lesions. We suppose that there are theoretically no limits for the size of the defect that can be treated with this technique. The limits are related to the technical execution of the procedure and the presence of intact cartilage shoulder around the implantation area. We undertook histological evaluation of the regenerating tissue, albeit after a short follow up of 12 months. Our findings were exactly in line with those we have published previously showing 50% hyaline cartilage and 50% fibrocartilage [13]. In the present arthroscopic study the number of biopsies for histological examination was low (n=2) for ethical reasons. The biopsies were only taken in those patients who clearly expressed their consent for the osteochondral biopsy for investigation purposes. In these 2 cases there was clear evidence of regenerated cartilage that was hyaline in one case and fibrocartilage in the other. The technical limit of the arthroscopic technique is the difficulty to treat lesions located on the patella, and posterior portion of femoral condyle. However, it should be noted that this limit is common to all arthroscopic techniques and could be partly solved only with development of new arthroscopic tools. In general, however, the autologous chondrocyte implant on three-dimensional scaffolds guarantees results comparable with the traditional ACI technique, but reduces the morbidity of the procedure and avoids the use of a periosteal flap with marked advantages from a biological and surgical point of view. The clinical and histological results of our first patients at medium follow up are encouraging. Our preliminary results suggest this method may be used for the treatment of large cartilage lesions, also in highly competitive athletes, but long-term and randomized controlled studies will be needed to confirm the reliability of this procedure.