ADIPOSE TISSUE

AN AMAZING SCAFFOLD FOR ONE-STEP BIOLOGICAL CARTILAGE REPAIR

– Written by Fabio Valerio Sciarretta, Italy

INTRODUCTION

Articular cartilage is avascular, aneural, alymphatic and has limited capacity for intrinsic repair or regeneration.

Over the last decades scientists have proposed several treatment options for the treatment of focal cartilage defects. For smaller cartilage lesions, these modalities include debridement, marrow stimulation through microfracture, abrasion, drilling and nano-fracture, advanced scaffold augmented marrow stimulation techniques (AMIC, BST-Cargel, GelrinC)), and osteochondral autograft transplantation (OATS) / mosaicplasty. For larger lesions, osteochondral allograft transplantation and autologous chondrocyte transplantation (ACI) have been introduced and appear to be highly effective.

The goal of any cartilage restoration procedure is to restore the articular surface by matching the histological, biochemical, and biomechanical properties of normal hyaline cartilage, improve patient symptoms and function, and prevent or at least slow down the progression of focal cartilage injuries to end-stage arthritis.

In an attempt to repair or regenerate articular cartilage in more recent years orthobiologic treatments with cells, mesenchymal stem cells (MSCs) and growth factors have become a new major interesting treatment choice for different pathologies regarding muscles, tendons, bone and especially cartilage.

MSCs are certainly the most investigated and used cell type and play a crucial role in most of the possible application protocols. Most recent discoveries have demonstrated that MSCs derive from perivascular cells named as "pericytes", located in the proximity of blood vessels[1,2]. Pericytes belong to the so called "perivascular niche"[3], where they live in a quiescent condition. When a vessel is damaged, as normally occurs during an injury, this event leads to the release of pericytes that from a quiescent phase pass to an activated phase, finally acquiring a MSCs phenotype. Activated MSCs start to release a cascade of bioactive molecules to counteract the overaggressive immune response[5] and trophic factors to establishing a regenerative microenvironment, promoting angiogenesis and stimulating proliferation of tissue specific progenitor cells[4,5].

The updated interpretation on MSCs activity, emphasises the fact that these cells show a very effective in vivo function and attributes their therapeutic action mostly to the demonstrated important paracrine and trophic actions that these cells are able to carry out. For this reason, MSCs have been recently defined as a "drugstore", since their ability to release a wide range of growth factors and cytokines in the surrounding microenvironment actually mimic the effect of a very powerful drug administered locally[5,6].

Orthobiologics include bone marrow aspirate concentrate (BMAC), Adipose Tissue-Derived Stroma Cells (ADSCs), platelet-rich plasma (PRP) and micronized allogeneic cartilage. Parallel to the use of orthobiologics, another promising strategy that has been recently introduced and extensively studied is the use of scaffolds alone or of scaffolds seeded with MSCs to enhance their differentiation in chondrocytes and form hyaline cartilage. In fact, if from one side scientists in the last years have been focusing on improving scaffold-based ACI, the literature[7] has seen and confirmed a constantly growing interest in looking for different solutions to regenerate the damaged articular cartilage by the use of different MSCs sources as a new powerful tool for three-dimensional scaffold augmentation surgical regeneration techniques.

Clinical applications of MSCs should meet the minimal criteria established by International Society for Cellular Therapy including[8] being plastic-adherent in culture conditions; expressing cluster of differentiation 105 (CD105), CD73, and CD90, lacking expression of CD45, CD34, CD14 or

CD11b, CD79 or CD19, and human leukocyte antigen-DR isotype (HLA-DR) surface molecules; and (3) possessing tri-lineage differentiation into osteoblasts, adipocytes and chondroblasts.

In Orthobiology, the innovative field of modern biomedical technology that provides biologic therapies for reconstructing damaged tissues, the utilised mesenchymal stem cells are in most cases obtained from bone marrow or adipose tissue.

In the past decade, adipose tissue has become a highly interesting source of adult stem cells for plastic surgery and regenerative medicine. More recently adipose tissue, that is provided of a consistent vasculature, has been progressively recognized as a smart source of these cells that can be easily collected in abundance with a lower invasiveness for the patient and fewer age-related restrictions. Adipose tissue grafts and ADSCs can be isolated from the upper arm, medial thigh, buttocks, trochanteric region, superficial deep abdominal depots and infrapatellar fat pad.

Subcutaneous adipose tissue is up today the first choice for cell isolation because it is easily accessible via liposuction, is relatively abundant in many patients, can be harvested by a minimally invasive procedure and can be safely and effectively transplanted to either in autologous or allogeneic setting[9-11]. This type of tissue provides an abundant source of stromal vascular fraction (SVF) cells for immediate administration.

The SVF fraction contains multiple non-cultured cell types, including preadipocytes, mesenchymal (MSCs) and endothelial progenitor cells (EPCs), fibroblasts, pericytes, and vascular smooth muscle cells. There are varieties of isolation systems commercially available for SVF isolation assuring a reproducible and consistent composition of heterogeneous cells[11-14]. Upon processing and administration, the adipose-derived SVF cells can differentiate into different tissue types, support neovascularization, replace cells and repair injured tissue.

Each adipocyte is completely surrounded by a capillary system, thus explaining how the amount of MSCs in adipose tissue is five time higher than the bone marrow's one. The identification of the stroma and the possibility to use this stromal vascular fraction with its high prevalence of stem/stromal cells for therapeutic uses, has made, as said, the adipose tissue a suitable source for clinical applications.

© Ayman Aref/NurPhoto/Getty Images

Image: Illustration.

Adipose tissue contains, in fact, a great number of stem cells (ADSCs), 500-fold greater than BM-MSCs. ADSCs represent a population of adult MSCs able to self-renew and multipotentially differentiate into adipocytes, chondrocytes, myocytes, osteoblasts, and play a key role in reconstructive or tissue engineering medicine, alone or in combination with biomaterials, growth factors or different types of scaffolds. According to published studies, cartilage repair with scaffold augmentation has improved clinical outcomes, radiological fill, and histological repair compared with microfracture alone. In particular, already in 2017, Pot et al[15] in a systematic review and meta-analysis on cellular and cellular scaffolds concluded that cartilage regeneration using ADSCs-seeded scaffolds improved regeneration compared to acellular scaffolds.

Collagen is one of the major components of cartilage ECM, and collagen-based scaffolds have been proven capable of retaining MSCs, providing high biocompatibility and a chondrogenic environment thus supporting functional cartilage regeneration. In particular collagen materials have been proven to provide a proper environment for cell invasion, facilitating cell colonisation through the pores of the material and finally enhancing chondrocytes differentiation. Thus, MSCs present in collagen scaffolds can maintain a chondrocyte phenotype in chondrogenic culture conditions and produce new collagen.

Cartilage defects repair by ADSCs has begun after that, in 2001 and 2002, Zuk et al[9,10] have been able to identify the processed lipoaspirates cells (PLA) and confirmed their ability, like MSCs, to differentiate toward chondrogenic lineages. The authors, in fact, proved that PLA cells were able to

express a unique set of CD markers, could synthetize cartilaginous matrix and express chondrogenic lineage genes.

Cell separation with minimal manipulation exploits mechanical steps, such as centrifugation, pressure, filtration and micro-fragmentation to separate cells from adipose tissue. Different closed systems based on these minimal manipulation technologies have been developed to harvest, concentrate and transfer the patient's own adipose tissue in the clinical setting meeting the strict regulations on MSCs use[16-21].

Nowadays, the literature has confirmed the good results obtained with ADSCs both as intra-articular injections in the hip and the knee as OA treatment and as orthobiologic complex scaffold reconstruction surgeries.

We personally developed the so-called LIPO-AMIC procedure[22], where the cartilage defect is, in the same operating procedure, subjected to careful debridement, accurate microfractures and finally collagen membrane coverage arthroplasty, where the especially studied 3Dmatrix bi-layer scaffold is carefully cut to the same size and shape of the defect to be treated and soaked for 10-15 minutes in the micro-fragmented adipose tissue transfer graft containing ADSCs and the stromal vascular fraction. To obtain the adipose tissue graft, a small quantity of lipoaspirate (about 50-80 ml), obtained from the abdomen adipose tissue reservoir, is treated in a closed system through a dedicated single-use kit available on the market for suction and subsequent processing (filtration and micro-fragmentation) and adipose tissue grafting, based on the mechanical disruption of the sample that enables to discard oil and debris and maintain cells niche and secretome and forward the graft in a syringe ready for use.

The LIPO-AMIC technique has been used for the treatment of knee symptomatic degree III and IV focal cartilage defects according to the classification of the International Cartilage Regeneration and Joint Preservation Society (ICRS) greater than 1 cm^2 in size (mean lesion size, 3,1 cm^2) and affecting the femoral condyles, medial or lateral, or the retrosurface of the patella or the femoral trochlea.

DETAILS OF SURGICAL TECHNIQUE

The patient undergoes one-step biological resurfacing procedure using microfractures, adipose tissue graft, ADSCs and collagen

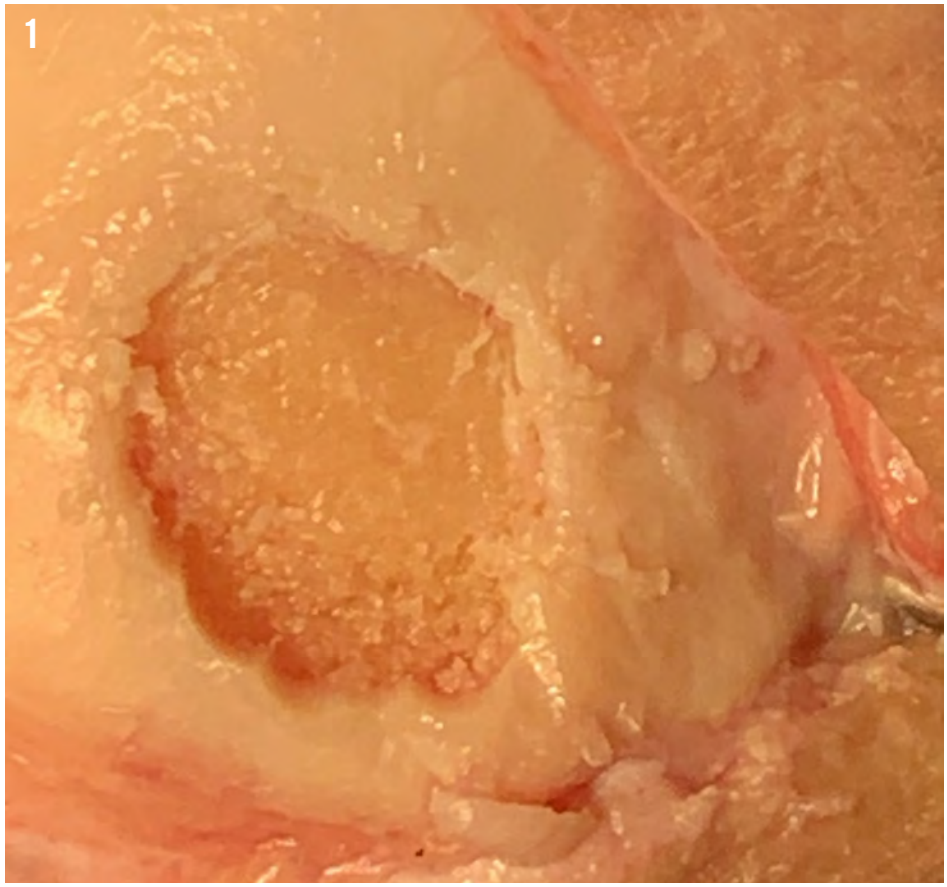

Figure 1: Large full thickness osteochondral defect of the articular surface of the patella.

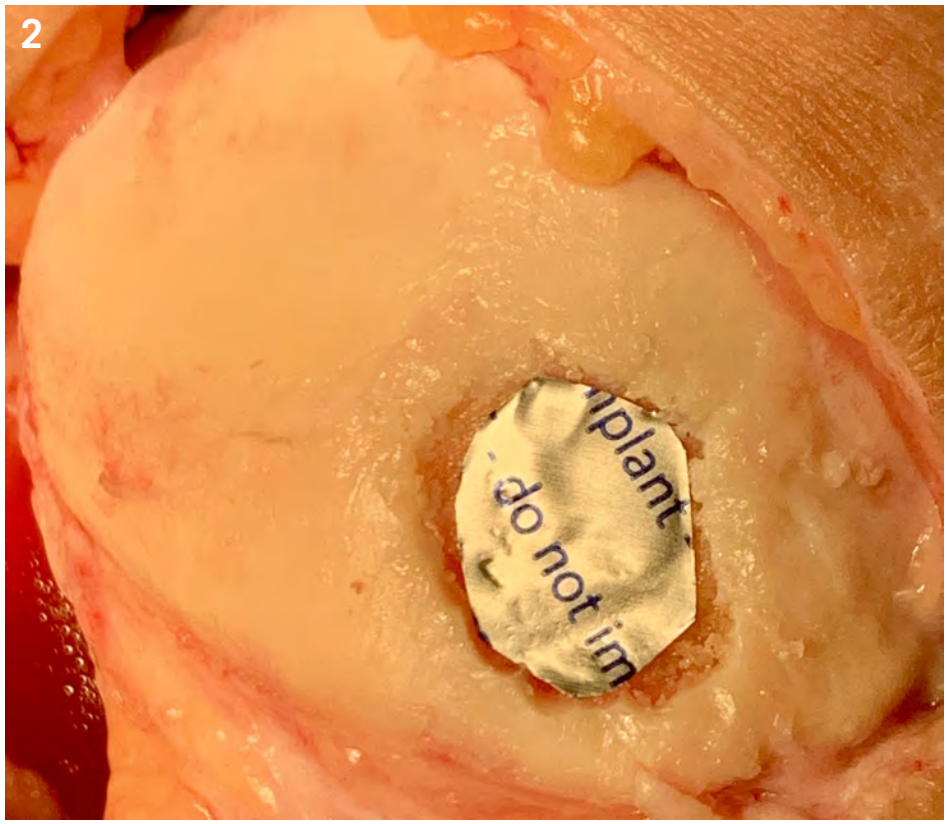

Figure 2: Creation of an exact figure of the full thickness osteochondral defect through the sterile aluminium template provided in the collagen membrane kit that will be used for the repair.

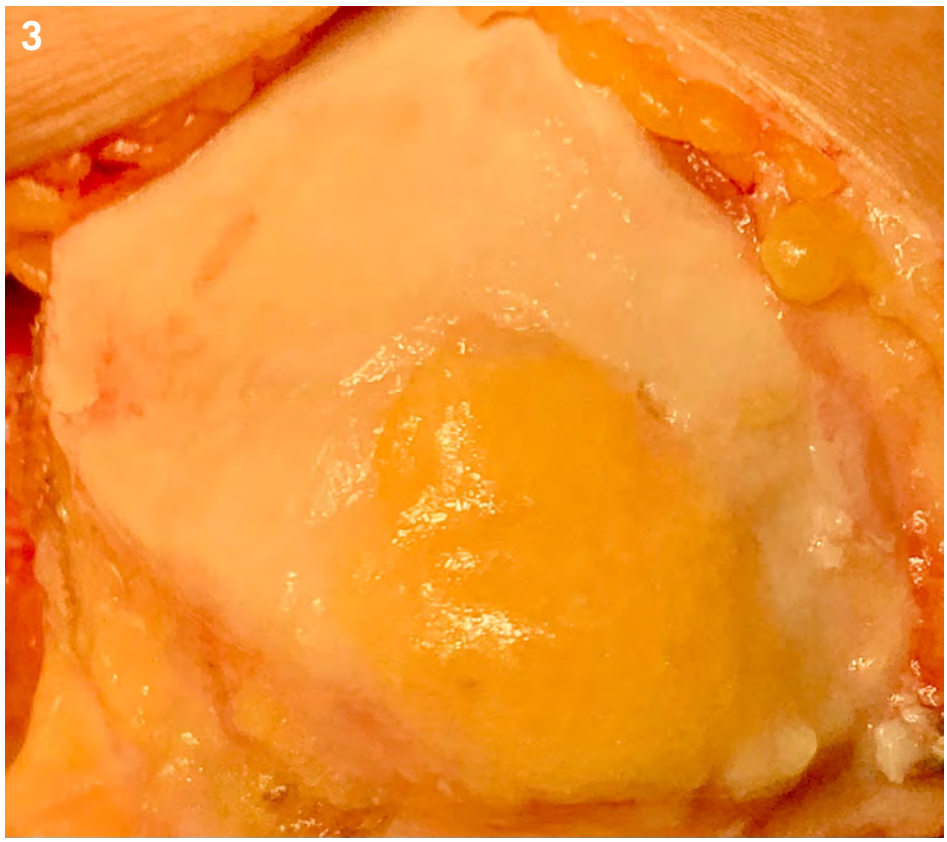

Figure 3: Biologic resurfacing arthroplasty of full thickness osteochondral defect of the articular surface of the patella by collagen membrane augmented with adipose tissue graft and ADSCs withdrawn form subcutaneous adipose tissue of the abdomen.

membrane coverage of full-thickness defect following arthroscopic confirmation of the presence of a defect suitable for such treatment (Figure 1).

All surgical procedures are performed in a single surgical step, under loco-regional anesthesia and after routine preparation of the sterile operating field.

The first phase of the procedure involves performing a normal diagnostic arthroscopic examination through the anteromedial and antero-lateral standard portals to confirm magnetic resonance imaging findings with respect to location and size of the lesions. During this phase, all the compartments of the knee are inspected, including the suprapatellar pouch, the medial and the lateral gutters, the patellofemoral joint, the intercondylar notch and the central pivot, the medial and lateral compartments, with particular probing and rating of the cartilage lining of the femoral condyles and the posterior horns of the medial and lateral menisci. Completed the diagnostic stage, which also ensures the site, the extent and depth of the chondral or osteochondral defect, the associated meniscal or synovial pathologies are treated during the standard arthroscopic surgical procedure.

Subsequently, under local anesthesia of the abdomen, after infiltration of Klein's solution, 50-80 ml of lipoaspirate are extracted by the simple method of lipoaspiration from the periumbilical adipose tissue, using the Lipogems® dedicated disposable kit available on the market for the suction and subsequent processing (filtration and micro-fragmentation) and grafting of adipose tissue, for the use of which we follow the instructions provided by the manufacturer. This device progressively reduces the size of the adipose tissue clusters, at the same time eliminating blood residues and oily substances with pro-inflammatory capacities, minimizing, thanks to the carrying out of the whole process inside physiological solution, the risks of damaging the mesenchymal stem cells.

Once the adipose graft harvesting process has been completed, the following step of the surgery is the repair of the focal chondral or osteochondral defect. Identified the full thickness cartilage defect, a thorough and extensive debridement of the defect is completed. At this stage, it is extremely important to focus in removing all the damaged and / or unstable cartilage till reaching the surrounding healthy cartilage tissue, creating solid and net margins. For this manouver, generally, straight or curve curettes, specific designed chondrotomes and an arthroscopic full radius cutter are used to carefully remove the cartilage degenerated or only partially still connected at the defect's edges. It is important to take care to remove always the calcified cartilage layer with a curette, also taking the utmost care in not to trespassing the subchondral plate.

Once completed the debridement and the careful grooming of the cartilage defect, the defect is measured and an exact figure is created through the sterile aluminum template provided in the collagen membrane kit that will be used for the repair of the full thickness cartilage defect (Figure 2). Thus, the exact imprint of the defect is cut out. Always remember to identify the side of the template, and then of the membrane, which will be placed in contact with the defect, in order to avoid obtaining a membrane of the wrong shape to apply on the defect. This same template will then used to prepare the scaffold used for the biological resurfacing.

While the cut membrane is soaked in the adipose tissue graft, we perform the microfractures in the subchondral bed of the lesion, using different angled drills, until reaching the subchondral bone. The microfractures are performed starting from the periphery of the defect proceeding concentrically toward the center of the defect itself, keeping a distance of 3-4 mm. between a perforation and the other and placing the utmost care in avoiding the convergence of a perforation in the other. Once reached the appropriate depth, typically 2-4 mm., leaking of some droplets of blood or fat is seen, which confirms the proper execution of the technique. After finishing the microfractures and verified to have penetrated the entire defect, it is important to carefully remove all the produced debris.

Completed the microfractures, the Chondro-Gide® collagen bilayer membrane is cut so that it has exactly the size and shape of the shape previously prepared. Once cut, the dry membrane is laid on the defect in order to make sure the size is perfect, even

in anticipation of the increase in volume of the membrane (10% -15%) as a result of its impregnation with the adipose tissue graft. This scaffold is a double layer membrane with a smooth side, waterproof and capable of retaining the cells, and the other wrinkled and porous.

Next step is the repair of the defect. This phase involves several sequential steps: first step is the injection of ADSCs in the defect bed. Then the enriched membrane is inserted into the joint to directly and accurately cover the defect. It is therefore crucial to be sure to apply the cell adhesive porous layer of the scaffold towards the bed of the defect in order to correctly favour cell entrapment.

The remaining part of the cells obtained from the adipose tissue is infiltrated on the site of the lesion and then, membrane and adipose graft and MSCs are sealed and secured to surrounding cartilage by use of fibrin glue (Figure 3).

The collagen membrane constitutes an ideal scaffold as its porous nature allows the cells to nest inside the cells, thus finding the most suitable environment for growth and differentiation. In this way, the membrane will perform a double action: in addition to representing the barrier capable of retaining the mesenchymal cells from the medullary blood, it constitutes a scaffold enriched and activated by the mesenchymal adipose cells to accelerate the process of chondrocyte development and differentiation.

After complete adhesion of the scaffold, the joint is several times completely flexed and extended and the stability of the applied membrane is checked. Completed the surgical procedure, a compression bandage is applied.

POST-OP PROTOCOL

The postoperative protocol foresees the progressive immediate partial loading assisted by crutches. In the first post-operative day, the patient starts immediately to regain joint ROM. The careful evaluation of the compliance characteristics of each patient argues in favor or not to use the passive joint mobilization (CPM), which allow to promote cartilage healing by favoring joint nutrition and reducing intra-articular adhesions formation. Range of motion and weightbearing protocols are differentiated depending on defect location, on the femoral condyle or the patello-femoral joint. In case of patello-femoral joint defects, progressive weightbearing with crutches is immediately allowed, limiting ROM from 0 degrees to 60 degrees of flexion for the first 3 weeks. In femoral condyles defects, weight bearing is restricted for the initial four weeks postoperatively, achieving unrestricted complete weightbearing generally by six weeks. Great importance is emphasised on early regaining of normal gait patterns. Once muscle strengthening is completed and joint movement is fully recovered, specific sport activity exercises are initiated under strict guidance of physiotherapist and trainer.

RESULTS

All patients with chondral injuries treated with the LIPO-AMIC technique have been followed prospectively and have been evaluated both clinically and by magnetic resonance imaging with progressive follow-ups at 6 and 12 months and have been available for follow-up assessments at 2 and 5 years postoperatively.

The clinical, and MRI follow-ups at 2 and 5 years have confirmed the good results with repair and regrowth of the cartilaginous tissue able to gain complete filling of the defects.

Evaluation of MR images progressively showed in the follow-up controls a significant reduction in the area of the chondral defect, with complete filling of the defect in the majority of cases, in the absence of signs of hypertrophy or bone oedema.

Parallel to the clinical study, we have also conducted an analysis of the fresh ADSCs isolated from adipose tissue lipoaspirate samples withdrawn to evaluate their biologics, integrity and viability to confirm their ability to guarantee safe and effective repair of articular cartilage defects. SVF cellular components from untreated lipoaspirate samples have been compared with corresponding cells deriving from lipoaspirate samples processed by micro-fragmentation, to evaluate cell composition, and preservation of viability. The outputs were characterized using multicolor Flow Cytometry (FC) analysis. We have found a consistent increase in the percentage of endothelial cells and pericytes in the processed samples by micro-fragmentation system compared to lipoaspirate untreated samples thus confirming that the mechanical withdrawal procedures may maintain a large cell population heterogeneity, preserving the niche tissue architecture.

CONCLUSIONS

In last years the literature has seen the publication of several phase I, II and now also III ADSCs trials that have confirmed the optimization of ADSCs preparations, good clinical results and safety of the procedures injecting or transplanting ADSCs using various scaffolds.

ADSCs implantation obtained by the single-step LIPO-AMIC procedure has provided encouraging outcomes with acceptable and more durable duration of symptom relief at midterm follow-up at 2 and 5-year years, thus representing a possible alternative to standard autologous chondrocyte implantation at lesser costs.

Cartilage regeneration by scaffold augmented techniques is nowadays a reality that holds significative promise for improving the results of cartilage repair and promoting the must of joint preservation, looking forward in the future to refine more and more increasingly mini-invasive and arthroscopic implantation techniques that together with novel next-generation biologics addition may improve the quality of tissue repair and faster the repair tissue integration and maturation processes.

Orthopaedic surgeons must, therefore, believe and get experienced in cartilage repair in order to fight joint degradation and OA development and keep patients on their legs to continue all daily and sport activities and move away the spectrum of a total joint procedure.

References

Available at www.aspetar.com/journal

Fabio Valerio Sciarretta M.D.

Chair of Education Committee of ICRS

Italian National Delegate and Chair of Sports Traumatology and Arthroscopy Committee of SICOT

Member of Education Committee of ISAKOS

Clinica Nostra Signora della Mercede, Rome, Italy

Contact: fvsciarretta@me.com