Aragonite-based Scaffold for the Treatment of Joint Surface Lesions in Mild to Moderate Osteoarthritic Knees:

Results of a Two-Year Multicenter Prospective Study
ABSTRACT

BACKGROUND: The treatment of knee Joint Surface Lesions (JSLs), i.e. chondral and osteochondral defects, has always been a challenge for surgeons, especially in the presence of osteoarthritis (OA). OA is considered a contra-indication to most cartilage repair techniques. Several regenerative approaches have been attempted with the aim of delaying or preventing joint replacement, with controversial results. Currently, there is a paucity of data on the use of single-step techniques, such as cell-free biomimetic scaffolds for the treatment of JSLs in OA knees.

PURPOSE: To present the 2-year follow-up clinical and radiologic outcomes after implantation of a novel cell-free aragonite-based biomimetic scaffold for the treatment of JSLs in patients with mild to moderate knee OA, in a multicenter prospective study.

STUDY DESIGN: Case series; Level of evidence: 4

METHODS: Eighty-six patients, 60 male and 26 female, relatively young (average age 37.4 ± 10.0 years) with mild-moderate knee OA and an average defect size of 3.0±1.7 cm², were recruited in 8 medical centers according to the following criteria: radiographic mild to moderate knee OA (Kellgren-Lawrence grade 2 or 3), with up to 3 treatable chondral/osteochondral defects (ICRS grades 3 and 4) on the femoral condyles or the trochlea, with a total defect size ≤ 7 cm²; no concurrent knee instability, severe axial malalignment, and systemic arthropathy. All patients were evaluated at screening and at 6, 12, 18 and 24 months after implantation, using the KOOS Subscales and IKDC-subjective score. Additionally, MRI evaluation was performed to assess the amount of cartilage defect fill at the repaired site.

RESULTS: Significant improvement in all KOOS subscales was recorded from basal (Pain: 49.6 ± 13.1, ADL: 56.1 ± 18.4, Sports: 22.8 ± 18.8, QoL: 23.5 ± 16.5, Symptoms: 55.4 ± 19.9) compared to the 24 months’ follow-up (Pain: 79.5 ± 21.1, p<0.001; ADL: 84.1 ± 21.4, p<0.001; Sport: 60.8 ± 31.9, p<0.001; QoL: 54.9 ± 30.4; p<0.001; Symptoms: 77.7 ± 21.2, p<0.001). IKDC-subjective score showed a similar trend and improved from 37.8 ± 14.7 at baseline to 65.8 ± 23.5 at 24 months (p<0.001). MRI evaluation showed a significant increase in defect filling over time, up to 78.7 ±
25.3% of surface coverage after 24 months. Treatment failure requiring revision surgery occurred in 8 patients (9.3%).

**CONCLUSION:** The use of an aragonite-based biomimetic osteochondral scaffold in patients with JSLs and mild to moderate knee OA provided significant clinical improvement at the 24-month evaluation, as reported by the patients. These findings were associated with good cartilage defect fill as observed on MRI.

**What is known about the subject:** There is an increasing number of relatively young patients affected by JSLs in OA knees. No joint preserving technology is currently available to delay progression of OA. Several studies aimed to treat young and active patients with mild-moderate OA using cartilage regenerative approaches, such as MACI, and have reported mixed results and relative high failure rates. Limited data is available on the use of cell-free osteochondral scaffolds for this indication.

**What this study adds to the existing knowledge:** This is the first multicenter study that reports results at 24 months - clinical and imaging outcomes - for a novel aragonite-based osteochondral scaffold used to treat JSLs in relatively young patients with mild-moderate knee OA (KL grade 2-3). The study includes the largest series of documented patients treated, without the influence of major concurrent treatments such as osteotomies. Final evaluation demonstrated a significant increase in all patient-reported outcomes. Additionally, MRI images demonstrated defect filling with repair tissue signals similar to hyaline-like cartilage and native subchondral bone. Histologic evaluation of a specimen taken from a treatment failure case who underwent total knee replacement, confirmed formation of articular hyaline cartilage and excellent subchondral bone healing.

**Keywords:** knee osteoarthritis; aragonite; scaffold; cartilage regeneration; osteochondral; Agili-C; preserving surgery.
INTRODUCTION

Osteoarthritis is a serious disease that causes premature morbidity and decreased function in the activities of daily living. Knee Joint Surface Lesions (JSLs), i.e. chondral and osteochondral defects, have always been a treatment challenge for surgeons, especially in the presence of joint degeneration. Osteoarthritis (OA) has been considered a contra-indication to cartilage repair procedures, due to a hostile joint environment where the increased concentration of pro-inflammatory molecules and catabolic agents may impair potential cartilage healing.

Symptoms of OA can range from joint stiffness and mild pain to severe pain. Other symptoms include swelling and limited range of motion. Osteoarthritis is considered by the FDA as a serious disease with an unmet medical need. According to FDA there is a true need to find strategies that will modify the progression of the disease and potentially change its natural course to prevent long-term disability. OA represents a global medical and socio-economic burden, and cannot be considered a disease of the “elderly population” anymore. The CDC reported that overall prevalence of OA in people older than 25 is 13.9%, and this percentage increases to 33.6% in the population over 65. OA is the third most rapidly rising condition associated with disability and it has become common to find young or middle aged patients presenting JSLs associated to unicompartmental or diffuse OA, which is characterized not only by the damage to the articular cartilage, but also by the impairment of subchondral bone, synovial membrane and peri-articular tissues. Due to lack of good joint-preserving treatment options, there has been an overwhelming increase in total knee replacement (TKR) procedures, associated with a decrease in the mean age of patients treated. This opens the problem of revision surgery in the near future, which will adversely impact these patients and the economy of health systems. Therefore, strategies for delaying, and even preventing, joint replacement in young and still active patients are of utmost clinical importance.
In the last 20 years researchers have attempted to treat JSLs in the presence of mild to moderate OA with regenerative treatments, resulting in unpredictable outcomes. There are a few studies investigating matrix-assisted autologous chondrocyte implantation (MACI) to treat medium-large focal defects in OA joints.\textsuperscript{19,21,29} Results reported at short term were fair,\textsuperscript{19,29} but gradual worsening and high failure rates were observed after a longer evaluation time period.\textsuperscript{1} Regardless of the outcomes, the use of MACI has some significant drawbacks, which are mainly related to the two-step surgical approach, which results in an inherent higher morbidity, regulatory and logistical issues of ex-vivo cell cultivation, and the high costs related to cell expansion. In addition, the inability to address the subchondral bone pathology, which is inherent to OA, limits the use of this procedure in a large number of patients.\textsuperscript{23}

For this reason, cell-free biomimetic scaffolds have been developed to promote regeneration of both the subchondral bone and overlying cartilage in medium to large JSLs. Such 3D scaffolds have the advantage of being an off-the-shelf product, thus always available for use in the OR. As such, they can be used to treat the JSL in a single step surgical procedure.\textsuperscript{20} Despite intense research in the fields of biomaterials and OA, only a few osteochondral scaffolds reached clinical use. There is a lack of data on their performance, especially when used to treat JSLs in the osteoarthritic environment.\textsuperscript{5,12}

The aim of this multicenter prospective study is to present the 2-year follow-up clinical and MRI outcomes following the implantation of a novel cell-free aragonite-based biomimetic scaffold in patients with mild to moderate knee OA. We hypothesized that the aragonite-based scaffold is safe and able to provide significant tissue healing associated with a meaningful clinical improvement in patient-reported outcomes, despite the presence of joint degeneration and hostile conditions.
MATERIALS AND METHODS

Ethical Approval

The present multicenter prospective clinical study was approved by the Hospital Ethic Committees and/or Internal Review Boards of each involved medical center. Informed consent was obtained from all participating patients.

Patients’ Selection

Each participating site (8 European hospitals) is a recognized cartilage disease treatment referral center. Patients’ enrollment took place in 2016-2017, and the following criteria were used for selection.

Inclusion criteria: 1) Patients aged 18 years or older with mild to moderate OA, according to X-ray (Kellgren-Lawrence score 2 or 3) at baseline; 2) up to 3 treatable JSLs (chondral/osteochondral, ICRS grade III-IV), located on the femoral condyles and/or the trochlea, 3) total treatable area ranging from 1 to 7 cm²; and 4) KOOS Pain score at screening between 30-65.

Exclusion criteria: 1) Bony defect depth deeper than 8mm (based on pre-op imaging and intra-operative findings); 2) articular cartilage lesions in the tibia or the patella, ICRS grade III or above; 3) previous surgery in the index knee within the past 12 months; 4) presence of ligamentous instability; 5) lack of functional remaining meniscus at the end of the procedure (i.e. subtotal or total meniscectomy; concomitant partial meniscectomy was allowed); 6) untreated malalignment in the index knee (more than 5 degrees varus or 5 degrees valgus); 7) any known history of tumor, infection, inflammatory arthropathy or crystal-deposition arthropathy in the index knee; 8) any known systemic cartilage and/or bone disorder, such as but not limited to chondrodysplasia or osteogenesis imperfecta; 9) Body mass index >35; 10) grade 4 osteoarthritis of the index knee according to the Kellgren- Lawrence scale; 11) History of any significant systemic disease, such as but not limited to, HIV, hepatitis or HTLV infection; known coagulopathies, that might compromise the patient's welfare.
**Scaffold Characteristics**

Agili-C™ scaffold (CartiHeal, Israel) is a porous, interconnected calcium carbonate (aragonite) implant derived from purified, inorganic coral exoskeleton. The scaffold is biphasic: the lower part of the implant (subchondral phase) is composed of inorganic aragonite. This part undergoes degradation and reconstitution to new subchondral bone by osteoclasts and osteoblasts. The upper, chondral phase of the implant undergoes mechanical processing to form a grid of micro-drilled channels. This design promotes bone marrow mesenchymal stem cells (MSCs) adhesion, differentiation and proliferation to chondrocytes and articular cartilage formation.\textsuperscript{16,17} The implants are sterilized by gamma irradiation. The implants are 10 mm in height and available in a range of diameters in order to properly match the lesion size: those used in this study ranged from 10 to 17.5mm in diameter.

**Surgical Technique**

The surgical technique is carried out while the patient is in a supine position under general or spinal anesthesia. A pneumatic tourniquet is applied to the proximal thigh. Initially, standard knee arthroscopy is performed to verify patient eligibility and to treat concurrent pathology (e.g. meniscal tears, loose body, etc.) when necessary. Depending on the size and location of the defect/s, a mini arthrotomy is performed using a medial or lateral parapatellar approach to expose the lesions. The implantation site is prepared using a proprietary surgical toolset (CartiHeal, Israel): the perpendicular aligner is positioned in the lesion center to verify perpendicularity to the articular surface. The aligner is used to place a K-wire in the defect, as the surgical instruments are cannulated and thread onto the K-wire to ensure correct preparation of the implantation site and accurate positioning of the implant. Using a motorized drill through a drill sleeve, a cavity of the required depth is prepared. Next a reamer is inserted to ensure that the correct depth was obtained and finally a shaper is introduced to achieve precise implant wall inclination. A 12mm deep cavity with perpendicular shoulders is thus created to allow press-fit fixation of the implant. The shaper and K-wire are removed and the cavity is washed out with saline solution to remove debris. Peripheral cartilage remnants are trimmed using a
proprietary cartilage cutter or surgical scalpel to ensure smooth edges and avoid invagination during implant insertion. The Agili-C™ implant is manually inserted into the prepared site: initially it is firmly pushed with the thumb and, subsequently, gently inserted (without impaction to avoid implant breakage) using a silicone-covered tamper, to its final position 2mm below the adjacent articular cartilage surface. The distal part of the implant must be firmly embedded in cancellous bone, or slightly under the subchondral bone plate, to guarantee optimal implant stability, as presented in Figure 1. When multiple Agili-C™ implants are used, it is important to keep a bone bridge of at least 5mm between implants to avoid impingement (Figure 1). Implant stability is tested by cyclic bending of the knee while the implant is under direct vision, both before and after tourniquet removal.
Figure 1: 48 years old female with mild OA (K/L 2) and 4.5cm² lesion on MFC with previous ACL reconstruction and partial meniscectomy; A) baseline AP X-ray, B) arthroscopic view of the lesion, C) implantation of 2 aragonite scaffolds (both 10mm in diameter), 2mm recessed relative to the articular surface and with a 5mm bridge between them, D) 12-month X-ray, E) 12-month MRI of the posterior implant, F) 12-month MRI of both implants, G) 24-month X-ray, H) 24-month MRI of the posterior implant, I) 24-month MRI of both implants showing cartilage regeneration, bone remodeling and bone/cartilage tidemark formation.

Post-operative Rehabilitation Protocol

The recommended rehabilitation program includes toe-touch weight bearing using crutches for 4 weeks, with then increasing partial weight bearing in order to reach full weight bearing after 6 weeks. During the first 48 hours, cryotherapy in combination with a continuous-passive-motion (CPM) device
are applied and continued for 3 weeks, together with active assisted range of motion exercises. Quadriceps isometric sets and electro-stimulation are initiated immediately post-surgery. Stationary cycling is introduced at 4 weeks, when knee flexion is about 100°. Hydrotherapy is advised immediately after suture removal.\textsuperscript{14} After approximately three months the patient will regain full active ROM and should introduce proprioceptive/balance activities, walking and resistance. Resistance muscle strengthening exercises can commence after three months coupled with a more demanding open kinetic chain (terminal leg extension) and closed kinetic chain (inner range quadriceps and modified leg press) exercises. Outdoor cycling activity are allowed only 6 months after the operation. Repetitive joint impact activities, such as ballgames, skiing or marshal-arts, were not allowed until first post-operative year.

**Clinical Evaluation**

All patients were evaluated before the surgical procedure and during the follow-up visits at 6, 12, 18 and 24 months. During these visits, they were clinically examined and questioned to assess their symptomatology, physical status and knee function using KOOS and IKDC-subjective scores.\textsuperscript{4} The primary endpoint of the study was the change in KOOS score from baseline to 24 months’ evaluation. Failure was defined as the need for re-intervention in the index knee during the follow-up period due to persisting symptoms.

**MRI Evaluation**

All patients underwent 1.5T or 3T MRI evaluation at 6, 12, 18 and 24-months follow-up. The following protocol was adopted: Field of view: 14cm; slice thickness 3-3.5mm; matrix 512 x 256 (or 384); Receiver bandwidth: 80-120Hx/pixel. Sequences: a) Coronal IW FSE no fatsat; TR ≥3000ms; TE = 30-40ms; b) Coronal PDW FSE with fatsat; TR ≥3000ms; TE =10-20ms; c) Sagittal IW FSE no fatsat; TR ≥3000ms; TE = 30-40ms; d) Sagittal PDW FSE with fatsat; TR ≥3000ms; TE = 10-20ms; e) Axial IW FSE no fatsat; TR ≥3000ms; TE = 30-40ms; f) Axial T2W FSE with fatsat; TR ≥3000ms;
Defect fill repair assessment (0-100%) was performed in a blinded manner by an independent radiologist, expert in cartilage repair assessment. On each MRI scan, 2-3 slices located within the implant on a sagittal scan and 2-3 slices located within the implant on a coronal scan were assessed. For each slice, the degree of cartilage defect volume fill was semi-quantitatively assessed in increments of 25% fill (i.e.: 0-24% fill, 25-49% fill, 50-74% and 75-100%). In case of multiple implants/defects a single range was calculated based on averaging all implants in the same joint.

**Histologic Evaluation**

Upon receipt of the harvested condyle, the specimen was cut using a microcutting technique (Exakt System) in order to isolate the implanted site. One portion was dehydrated in alcohol solutions, cleared in xylene and embedded in PMMA resin block, that was then cut longitudinally to obtain 3 sections for paragon stain.

The other portion of the specimen was rinsed and decalcified in ethylene diamine-tetra-acetic acid solution (EDTA). After complete decalcification and dehydration in alcohol solutions of increasing concentration, the specimens were cleared in xylene and embedded in paraffin. The embedded specimens were then longitudinally cut (5 μm thickness ± 0.5μm) using a microtome (MICROM®, France). Five central full-length serial sections per block were prepared and stained with modified Masson’s Trichrome (MT), safranin Haematoxylin Eosin (HE) and safranin-O-Fast Green (SOFG).

Two sections were used for immuno-histochemical determination of collagen type I, and collagen type II presence.

**Statistical Analysis**

All continuous data were expressed as mean and standard deviation; categorical variables were expressed as frequency and percentages. Differences among times were explored with repeated measure ANOVA and mixed effect models. Multiple comparison p value were Bonferroni corrected.
For all tests, $P<0.05$ was considered significant. All statistical analysis was performed with SPSS, version 19.0 (IBM, Armonk, New York).

## RESULTS

### Patients Demographics

86 patients, 60 men and 26 women, were treated in the study. Mean age was $37.4 \pm 10.0$, mean BMI $26.1 \pm 3.5$, and lesion size averaged $3.0 \pm 1.7 \text{ cm}^2$. Demographic data is summarized in Table 1. Six patients were lost to follow-up at the 24-month evaluation.

### Table 1: Demographic Data of the patients included in the study

<table>
<thead>
<tr>
<th>Total number of Patients</th>
<th>86</th>
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<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>$37.4 \pm 10.0$</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>$26.1 \pm 3.5$</td>
</tr>
<tr>
<td>Sex</td>
<td>60 M (69.8%)/ 26 F (30.2%)</td>
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<tr>
<td>Previous surgery in the affected knee</td>
<td>48 (55.8%)</td>
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</table>
| ICRS grade               | Grade 3: 21 (24.4%)  
                          | Grade 4: 65 (75.6%) |
| Lesion size in cm$^2$ (mean ± SD) | $3.0 \pm 1.7$ |
| Lesion location          |     |
| Medial femoral condyle   | 44 (51.2%) |
| Lateral femoral condyle  | 15 (17.4%) |
| Trochlea                 | 13 (15.1%) |
| Multiple sites           | 14 (16.3%) |
| K/L grade                | Grade 2: 75 (87.2%)  
                          | Grade 3: 11 (12.8%) |
| Concomitant procedures   | 19 pts (22.1%)  
                          | 2 HTO, 8 partial meniscectomy, 1 meniscal suture, 4 debridement of other superficial lesions (ICRS grade I or II), 3 loose body removal, 1 synovial plica removal |
Clinical Scores Trend

A statistically significant improvement in each of the clinical scores used from baseline to the 24-month follow-up was recorded (Table 2).

KOOS subscales showed significant increase from baseline to the 6-month evaluation (p<0.001 in all cases; all values reported in Table 2), with further improvement at 12, 18 and 24-month follow-up. (Table 2; Figure 2A-B). IKDC-subjective score showed a similar trend, with a significant increase from baseline to the 6 months’ evaluation (37.8±14.7 vs 55.4±21.5 respectively; p<0.001), followed by further significant improvements at 12, 18 and 24-months (Figure 3).

Table 2: Summary of Clinical scores and MRI evaluation at baseline, 6, 12, 18 and 24-months follow-up (data expressed as mean ± SD; * significant difference compared to baseline with p<0.001; ** significant difference compared to 6mo with p=0.01; *** significant difference compared to 6mo with p<0.001).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>12 months</th>
<th>18 months</th>
<th>24 months</th>
<th>p (24 m vs. basal)</th>
</tr>
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<tr>
<td>KOOS Pain</td>
<td>49.6±13.1</td>
<td>73.0±21.1*</td>
<td>77.5±19.6</td>
<td>78.1±21.1</td>
<td>79.5±21.1***</td>
<td>&lt;0.001</td>
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<tr>
<td>KOOS ADL</td>
<td>56.1±18.4</td>
<td>78.7±20.9*</td>
<td>82.5±18.9</td>
<td>83.5±20.3</td>
<td>84.1±21.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>KOOS Sport</td>
<td>22.8±18.8</td>
<td>48.1±29.5*</td>
<td>55.5±29.9</td>
<td>56.0±31.9</td>
<td>60.8±31.9***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>KOOS Symptoms</td>
<td>55.4±19.9</td>
<td>71.9±21.7*</td>
<td>75.9±19.8</td>
<td>76.1±22.0</td>
<td>77.7±21.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>KOOS QoL</td>
<td>23.5±16.5</td>
<td>44.7±27.6*</td>
<td>48.7±26.3</td>
<td>52.4±27.7</td>
<td>54.9±30.4***</td>
<td>&lt;0.001</td>
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<tr>
<td>KOOS Overall</td>
<td>41.5±14.3</td>
<td>63.3±21.7*</td>
<td>68.0±20.9</td>
<td>69.2±22.8</td>
<td>71.4±23.6***</td>
<td>&lt;0.001</td>
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<td>(average of all 5</td>
<td></td>
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<tr>
<td>subscales)</td>
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<tr>
<td>IKDC</td>
<td>37.8±14.7</td>
<td>55.4±21.5*</td>
<td>62.2±20.6**</td>
<td>63.6±21.6</td>
<td>65.8±23.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MRI Defect Fill</td>
<td>N/A</td>
<td>63.7±29.1</td>
<td>70.3±28.6</td>
<td>77.7±26.0</td>
<td>78.7±25.3</td>
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</table>
Figure 2: A) KOOS subscale trend at baseline, 6, 12, 18 and 24-month follow-up (mean values and confidence interval are depicted for each time-point).

B) Overall KOOS score trend at baseline, 6, 12, 18 and 24-month follow-up (box-and-whiskers plot showing: median, Q1-Q3 interquartile range, Min and Max values)
Figure 3: IKDC-subjective score trend at baseline, 6, 12, 18 and 24-month follow-up (box-and-whisker plots showing: median, Q1-Q3 interquartile range, Min and Max values)

Adverse Events and Revisions

Thirty-six patients experienced adverse events (AE) during the study duration. In 12 patients the AE were defined as unrelated. Sixteen patients experienced knee swelling and pain and were treated conservatively, except one patient that underwent synovectomy (without implant manipulation).

Eight patients (9.3%) underwent implant removal during the two-year. Reasons for implant removal were: procedure-related infection in 2 cases; lack of scaffold integration in 5 cases; progression of OA in the patello-femoral compartment in 1 case (this patient underwent TKR 14 months after scaffold implantation on the MFC).
Histology

The specimen explanted from the patient who underwent TKR was sent to an independent lab (NAMSA, France) for GCP histological analysis which revealed: a) newly formation of articular hyaline cartilage on most of the surface of the implant, with a marked grade of collagen type II, lack of collagen type I and homogenous proteoglycan expression; b) restoration of the subchondral bone plate with trabecular architecture and integration within the surrounding native bone, through osteoconduction and osteo-transduction, and formation of a well defined tidemark (Figure 4).
**Figure 4:** Histologic evaluation of the explanted specimen. A) Paragon stain – regeneration of new articular cartilage and subchondral bone, through implant remodeling, B) Safranin-O-Fast Green stain - indicating on high level of proteoglycan content in the newly formed cartilage, C) Collagen type II marker – indicating on hyaline cartilage formation, D) Safranin Hematoxilin Eosin stain – indicating on absent of inflammatory reaction, E) Masson trichrome - general morphology assessment of the repaired tissue, F) Collagen type I marker – indicating on absent of Coll I in the cartilage and presence of Coll I in the repaired bone, G) Paragon – indicating the newly formed tidemark and calcified cartilage, H) Paragon stain - demonstrating the osteo-induction and osteo-transduction (aragonite/bone remodeling), I) the harvested condyle (upper image) and cross section at the center of the implant (lower image) – white arrows indicate the newly formed articular cartilage, black arrows indicate implant/bone remodeling.

**MRI Evaluation**

All patients except those lost to follow-up and treatment failures performed the 24-month evaluation. A significant increase in the area of defect covered by cartilage regrowth was observed (Figure 1). As early as 6 months post implantation significant defect fill was observed (63.7 ± 29.1); the degree of defect fill continued to improve at the 12 and at 18 months’ evaluation (70.3 ± 28.6 and 77.7 ± 26.0 respectively) and reached a maximum after 24 months (78.7 ± 25.3; p<0.001 vs 6 months’ score; Table 2).

**DISCUSSION**

The main finding of the study is that an aragonite-based scaffold may provide significant clinical improvement in patients with JSLs (chondral and osteochondral defects) in mild to moderate OA knees. Evidence of MRI defect fill at the scaffold’s surface supports the positive clinical outcome. KOOS and IKDC-subjective scores improved after 6 months and continued to further increase during the subsequent follow-up visits at 12, 18 and 24 months. The patients included in the current study are often seen in clinical practice, and represent an undisputed clinical challenge: first, because these patients are relatively young and present high functional needs and expectations, and secondly due to the biology and the biomechanics of their knees, which is already affected by the presence of OA as well as the inherent impairment involving all intra and extra-articular tissues.
OA has been recognized as a severe and disabling condition, with a high impact on the global society. Its incidence is constantly increasing, including the young and middle-aged population, for whom functional limitations in work and sports present a psychological burden deeply affecting their quality of life. \(^{22}\)

OA is a multifaceted disease, with various clinical etiopathology, ranging from inflammatory to more mechanical variants. \(^{24}\) The course of OA is often unpredictable and current knowledge does not enable sufficient profiling. \(^{9}\) Disease modifying treatments, aimed at delaying joint replacement, are essential in order to avoid an overload of “young” prosthetic patients who will require one or even multiple revision surgeries during their life. \(^{22,26}\)

In this complex scenario, JSLS treatment is considered a salvage option in order to avoid more aggressive procedures such as cartilage regenerative strategies which are influenced by the osteoarthritic environment characterized by high concentrations of pro-inflammatory cytokines, metalloproteinases and other catabolic agents. OA is considered a possible contra-indication for the use of MACI, \(^{29}\) but, when employed as a salvage procedure, it has showed some encouraging outcomes: Minas et al. \(^{29}\) documented significant and stable clinical results with a low percentage of failures in an 11 year follow-up of a cohort of 153 patients affected by early OA (i.e. Ahlbach grade 0-1). Kreutz et al. \(^{19}\) treated a cohort of 19 patients who presented a more advanced level of OA (Kellgren-Lawrence 2 or 3), and revealed satisfactory outcomes in the middle term evaluation (4 years). Interestingly, in both studies a number of patients were treated with concurrent osteotomy, which in itself may have played a significant role in the clinical outcome. On the other hand, disappointing outcomes were recently published by Andriolo et al. \(^{1}\), who documented a cumulative 59% failure rate in 41 patients with Hyalograft-C (Kellgren Lawrence grade 2-3) after 15 years. More advanced OA is likely associated with lower long-term success rates of MACT \(^{1}\). Beyond the unavoidable progression of OA which could damage or induce apoptosis of the transplanted cells, there are some limits of the MACT technique itself: first, chondrocytes harvested from OA knees may not have the same biologic properties of those taken from healthy knees \(^{27}\) and, secondly, the
impairment in subchondral bone, which may impact the graft survival. Biochemical and physical alterations in the subchondral bone region are always present in OA knees, and therefore a “surface” treatment like MACT can be negatively affected by these pathologic changes in the long term. To overcome this drawback, and also other flaws of MACT such as the need for two surgical steps and cell manipulation, biomimetic cell-free scaffolds have been introduced. These biomaterials have been developed with the aim of promoting tissue regeneration both at the level of the subchondral bone and the cartilage layer, without the need of cell expansion, by recruiting resident autologous mesenchymal stem cells. The mechanism of action consists of providing a micro-environment where cells can differentiate and produce extra-cellular matrix. The scaffolds have different layers, that promote concurrent restoration of subchondral bone and cartilage. Despite extensive pre-clinical research in the field of biomaterials, just a few osteochondral scaffolds have reached clinical practice and, in spite of promising results demonstrated in the animal model, their regenerative potential in the human setting has thus far showed less favourable outcomes, especially concerning the subchondral bone. The first scaffold available was a bi-layered cylindric implant made of a polylactide-coglycolide copolymer, for which controversial results were shown. Among the few case series published, Dhollander et al. recorded a failure rate of 20% (3 out of 15 patients) at 1-year follow-up and biopsies showed fibrous vascularized repair tissue. The other scaffold available was introduced more than 10 years ago and is a 3-layered implant consisting of a blend of hydroxyapatite and type I-collagen at different percentage within the various layers. Two trials investigated the use of this scaffold in early OA patients. Condello et al. documented a success rate of only 69% in a cohort of 26 patients evaluated up to 3 years, whereas Sessa et al. evaluated 22 patients and reported satisfactory results up to 5 years, with a cumulative failure rate of 16.6%. Despite these somewhat positive results, MRI and CT evaluations showed slow and limited subchondral bone healing, which could impact the long-term outcomes, especially in complex patients. Literature therefore has a poor amount of data concerning osteochondral scaffolds in patients affected by OA. The scaffold tested in the present trial is a bi-phasic implant, composed of inorganic calcium carbonate, i.e. aragonite, which
is a natural biomaterial with a three-dimensional microarchitecture similar to human bone, including a comparable inter-connected pore network, and a crystalline form of calcium carbonate (CaCO₃) analogous to physiological hydroxyapatite. Aragonite is derived from coral exoskeleton and its application in orthopaedics as a bone substitute is well documented. The unique feature of this novel osteochondral scaffold is in fact its ability of restoring the subchondral bone as documented by extensive in vitro studies which showed not only osteoinductive and osteoconductive capabilities, but also unique osteotransductive properties, i.e. the formation of bone through direct deposition of bone trabeculae on the scaffold material. The chondrogenic potential of the superficial phase of the scaffold has been studied in another ex-vivo trial, and these findings were also confirmed in the goat model where the scaffold was able to restore the entire osteochondral unit even in extremely large defects. The findings of the present study further support the regenerative potential of the scaffold, even in complex patients such those affected by mild-to-moderate OA. The MRI analysis revealed good defect filling at the cartilage layer and good subchondral bone reconstruction, as the scaffold gradually degraded over time. These findings were also confirmed by a histologic examination conducted on the explanted specimen: the regeneration of the osteochondral unit was proved by the presence of newly formed hyaline cartilage, rich in collagen type II and proteoglycans and lack of collagen type I, as well as subchondral bone plate restoration with newly formed trabecular bone tissue associated with an ongoing osteotransduction process. Moreover, the regenerated tissues were well integrated within the adjacent native cartilage and bone, which also supports the potential of the aragonite scaffold.

The results hereby presented are particularly relevant for a number of reasons. First, due to the paucity of data on osteochondral scaffolds, and second because this is the only multicenter trial available on the use of an osteochondral scaffold in OA knees, with the highest number of patients included to date (86). Another major point is that, as opposed to other reports, only a small number of patients of the present series (19) underwent concurrent surgery (only two were major procedures, i.e. HTO), thus allowing better assessment of the performance of the scaffold itself without the bias of confounding
factors. Last but not least, positive outcomes were reported in the most challenging category of patients, those with KL grade 3, for whom there are minimal data in published literature. Despite the lower defect fill observed in MRI, clinical scores markedly improved and no failures occurred in this subgroup. These findings are particularly relevant since they further support the role of biologic procedures as a “joint preservation” approach for patients not ready to receive metal resurfacing. Even in the presence of moderate OA, osteochondral regenerative procedures may provide good outcome and contribute in postponing joint replacement. Future research should try to understand patient-related prognostic factors in order to optimize the clinical indications and select patients with higher chances of success.

The present study suffers from a limitation due to the absence of a matched control group. Moreover, the small amount of histologic data must be acknowledged, since only one specimen was fully processed and analyzed from a patient who received a TKR due to progression of OA in the patello-femoral compartment.

The Agili-C™ implant for the treatment of ICRS grade III-IV defects in osteoarthritic knees provides promising clinical and radiologic outcomes at 2-year evaluation, suggesting that the aragonite-based scaffold is capable of promoting satisfactory healing of the osteochondral unit, despite a hostile joint environment. Even the failure rate (9.3%) was acceptable given the complex category of patients treated. In addition, this is a single stage procedure with a less expensive off-the-shelf implant than other available treatments. Randomized controlled studies compared to surgical standard of care are required in order to assess if this implant is a superior treatment option. Additionally, longer-term evaluation is required in order to assess the durability of the outcomes, to understand if it has the potential to delay joint replacement and to establish if it can be considered as a disease modifying treatment.
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**LEGEND**

**Table 1**: Demographic Data of the patients included in the study

**Table 2**: Summary of Clinical scores and MRI evaluation at baseline, 6, 12, 18 and 24-months follow-up (data expressed as mean ± SD; * significant difference compared to baseline with p<0.001; ** significant difference compared to 6mo with p=0.01; *** significant difference compared to 6mo with p<0.001).

**Figure 1**: 48 years old female with mild OA (K/L 2) and 4.5cm² lesion on MFC with previous ACL reconstruction and partial meniscectomy; A) baseline X-ray, B) arthroscopic view of the lesion, C) implantation of 2 aragonite scaffolds (both 10mm in diameter), 2mm recessed relative to the articular surface and with a 5mm bridge between implants, D) 12-month X-ray, E) 12-month MRI of the posterior implant, F) 12-month MRI of both implants , G) 24-month X-ray, H) 24-month MRI of the posterior implant, I) 24-month MRI of both implants showing cartilage regeneration, bone remodeling and bone/cartilage tidemark formation.

**Figure 2**: A) KOOS subscale trend at baseline, 6, 12, 18 and 24-month follow-up (mean values and confidence interval are depicted for each time-point).
B) Overall KOOS score trend at baseline, 6, 12, 18 and 24-month follow-up (box-and-whisker plots showing: median, Q1-Q3 interquartile range, Min and Max values)

Figure 3: IKDC-subjective score trend at baseline, 6, 12, 18 and 24-month follow-up (box-and-whisker plots showing: median, Q1-Q3 interquartile range, Min and Max values)

Figure 4: Histologic evaluation of the explanted specimen. A) Paragon stain – regeneration of new articular cartilage and subchondral bone, through implant remodeling, B) Safranin-O-Fast Green stain - indicating on high level of proteoglycan content in the newly formed cartilage, C) Collagen type II marker – indicating on hyaline cartilage formation, D) Safranin Hematoxilin Eosin stain – indicating on absent of inflammatory reaction, E) Masson trichrome - general morphology assessment of the repaired tissue, F) Collagen type I marker – indicating on absent of Coll I in the cartilage and presence of Coll I in the repaired bone, G) Paragon – indicating the newly formed tidemark and calcified cartilage, H) Paragon stain - demonstrating the osteo-induction and osteo-transduction (aragonite/bone remodeling), I) the harvested condyle (upper image) and cross section at the center of the implant (lower image) – white arrows indicate the newly formed articular cartilage, black arrows indicate implant/bone remodeling.