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

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. Under a pathogenetic point of view, there is a continuity between JSLs and OA: after the onset of a JSL there is an increased risk of developing OA in the same joint over time. OA is currently considered as a serious disease with an unmet medical need and it cannot be considered a disease of the “elderly population” anymore: in fact, in current practice it has become common to diagnose JSLs in relatively young or middle aged patients already presenting signs of mild-moderate OA, and the presence of these cartilage defects has been associated with disease severity and considered a predictor of joint replacement at middle-term.

In the last 20 years researchers have attempted to treat JSLs associated to mild or moderate OA with different strategies, from simple arthroscopic debridement to microfracturing, resulting in unsatisfactory outcomes in the majority of cases, due to the impossibility of these treatments to modify the course of the disease. In more recent years, there have been also a few studies investigating matrix-assisted autologous chondrocyte implantation (MACI) to treat medium-large focal defects in OA joints: results reported at short term were fair, but gradual worsening and high failure rates were observed after a longer evaluation time period. 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.
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. 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.

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 (CaCO$_3$) analogous to physiological hydroxyapatite. Aragonite is derived from coral exoskeleton and its application in orthopaedics as a bone substitute is well documented. Although no pre-clinical study compared the aragonite to other similar osteochondral scaffolds, pre-clinical results were considered encouraging to adopt it in clinical practice: in fact, the unique feature of this novel scaffold is its ability of restoring the subchondral bone as documented by extensive in vitro studies, which showed not only its 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 articular phase of the scaffold has been studied in another ex-vivo trial, and then 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 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 chondral/osteoochondral defects in the context of mild to moderate knee OA. We hypothesized that the aragonite-based scaffold is safe and able to provide significant tissue regeneration.
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 Ethics 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, before signing the consent for study participation, all the patients were informed on the presence of degenerative changes in their knee and inherent implications. All patients were counselled about the possible alternative treatments, and, patients with Kellgren-Lawrence grade 3 were informed specifically about the option of arthroplasty. With regards to patients’ selection, the following criteria were used.

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. The choice of the aforementioned KOOS Pain interval was made to avoid inclusion of patients with too poor symptoms to justify surgical treatment at baseline (KOOS pain > 65) or patients with very intense pain (KOOS Pain <30), attributed mainly to the underlying OA, that were supposed to have unrealistic chances of a satisfactory outcome from the regenerative treatment proposed. Similar criteria were also used in other studies.23,24

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, characterized by macroporosity (pores’ diameter: 100-200 µm) that promotes vascular tissue ingrowth. 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 (Fig. 1). This design promotes bone marrow and synovial mesenchymal stem cells (MSCs) adhesion, differentiation and proliferation to chondrocytes thus leading to articular cartilage formation, as shown in a previous ex-vivo study investigating the mechanisms of cartilage regeneration promoted by the aragonite scaffold. The implants are sterilized by gamma irradiation, 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 17.5 mm in diameter.
Fig 1: The Agili-C aragonite-based scaffold. The micro-drilled layer represents the surface of the scaffold, which should be placed 2 mm below the adjacent surrounding cartilage by press-fit manner.

**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: testing on the animal model have shown that placing the implant slightly below the articular surface increases the overall stability of the scaffold, which is this way firmly embedded in the cancellous bone, and also enhances cartilage healing due to reduction of shear stresses exerted at the surface of the scaffold (Figure 2).

When multiple Agili-C™ implants are used, it is important to keep a bone bridge of at least 5mm between implants to avoid impingement. Implant stability is tested by cyclic bending of the knee while the implant is under direct vision, both before and after tourniquet removal.
Fig. 2: A 32 year-old female with mild OA and an osteochondral defect on her LFC. The patient was treated with a single aragonite-based implant. A) Baseline MRI; B) intraoperative view on the defect; C) Agili-C implantation (note the positioning – 2mm below articular surface); D) 6 months’ MRI; E) 12 months’ MRI; F) 24 months MRI; G) 6 months X-ray; H) 12 months X-ray; I) 24 months X-ray. Overall KOOS score trend: 29.1 at baseline; 50.3 at 6m; 72.9 at 12m; 76.8 at 18m; 93.6 at 24m.

Post-operative Rehabilitation Protocol

The rehabilitation program was defined based on the authors’ previous experience using another osteochondral scaffold, and included 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. 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 were allowed 6 months after the operation. Jogging and running activities could be resumed at about 8 months from surgery, whereas repetitive joint impact activities, such as ballgames, skiing or martial arts, were allowed after one 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. The primary endpoint of the study was the change in KOOS score from baseline to 24 months’ evaluation.

Failures were defined as patients who underwent scaffold removal for any reason during the follow-up period. Any other re-intervention on the index knee was considered as “adverse event” and has been described in the dedicated paragraph in the Results section.

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; TE = ≥70ms; g) Sagittal T1W no fatsat; TR = 600-800; TE = 10-20ms; h) Oblique PDW FSE with fatsat; TR ≥3000ms; TE = 10-20ms oriented perpendicularly to the scaffold.

Defect fill repair assessment (0-100%) was performed in a blinded manner by an independent radiologist, expert in cartilage repair assessment. For condylar defects, all the aforementioned sagittal and coronal sequences were evaluated, whereas for trochlear defects axial and sagittal scans were considered. On each MRI sequences, 2-3 slices located within the implant 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%). The defect fill in each sequence was therefore calculated by averaging the scores of each single slice, and the overall value of defect fill was the average among the scores of all the analyzed sequences. 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 ± 10.0, mean BMI 26.1 ± 3.5, and lesion size averaged 3.0 ± 1.7 cm². Demographic data is summarized in Table 1. Six patients were lost to follow-up at the 24-month evaluation.

<table>
<thead>
<tr>
<th>Total number of Patients</th>
<th>86</th>
</tr>
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<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>37.4 ± 10.0</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>26.1 ± 3.5</td>
</tr>
<tr>
<td>Sex</td>
<td>60 M (69.8%)/ 26 F (30.2%)</td>
</tr>
<tr>
<td>Previous surgery in the affected knee</td>
<td>48 (55.8%)</td>
</tr>
</tbody>
</table>
| ICRS grade               | Grade 3: 21 (24.4%)
                          | Grade 4: 65 (75.6%) |
| Lesion size in cm² (mean ± SD) | 3.0 ± 1.7 |
Lesion location

<table>
<thead>
<tr>
<th>Location</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial femoral condyle</td>
<td>44 (51.2%)</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>15 (17.4%)</td>
</tr>
<tr>
<td>Trochlea</td>
<td>13 (15.1%)</td>
</tr>
<tr>
<td>Multiple sites</td>
<td>14 (16.3%)</td>
</tr>
</tbody>
</table>

K/L grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Count (Percentage)</th>
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<tbody>
<tr>
<td>2</td>
<td>75 (87.2%)</td>
</tr>
<tr>
<td>3</td>
<td>11 (12.8%)</td>
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</tbody>
</table>

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 6-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 3). 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 4).

A subgroup analysis was performed comparing clinical outcomes of KL grade 2 vs KL grade 3 patients and no significant difference were observed in the scores considered at any follow-up evaluation.

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>
</thead>
<tbody>
<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></td>
<td>15 ± 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>
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<tr>
<td>KOOS ADL</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 Sport</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 Symptoms</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>
</tr>
<tr>
<td>KOOS QoL</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>
</tr>
<tr>
<td>KOOS Overall (average of all 5 subscales)</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>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>
</tbody>
</table>

| MRI Defect Fill [%] | N/A    | 63.7 ± 29.1 | 70.3 ± 28.6 | 77.7 ± 26.0 | 78.7 ± 25.3 |

**Figure 3:** 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 4: 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 Failures

Thirty-six patients in total (including those defined as “failures”) experienced adverse events (AE) during the study duration:

- 15 patients experienced episodes of knee swelling and pain and were treated conservatively by decrease of physiotherapy/working activities, local cryotherapy and oral non-steroidal anti-inflammatory drugs;

- 3 cases presented knee stiffness: 2 patients required knee manipulation under narcosis whereas 1 patient required prolonged rehabilitation;

- 1 patient had delayed surgical wound healing, managed by seriated dressings which allowed complete healing of the wound;
- 3 patients complained about onset of knee pain that was attributed to overload during physiotherapy. All were managed by adapting the rehabilitation program and resolution of pain was achieved;
- 2 patient had knee trauma during follow-up: in one case a medial meniscus tear occurred and the patient underwent partial meniscectomy, whereas in the other case an hyperextension trauma caused low grade muscle strain that was managed conservatively;
- 1 patient underwent loose body removal (bony fragment) that caused occasional locking episodes;
- 1 patient suffered from patellar tendinitis that was managed conservatively;
- 1 patient complained about persisting quadriceps weakness;
- 1 patient showed synovial hypertrophy and exuberant intra-articular scar tissue, and underwent arthroscopic synoviectomy and scar tissue removal

Eight patients (9.3%) underwent implant removal during the two-year and were considered failures. In all cases, relapse of severe knee pain with swelling and consequent motion limitation was observed. Reasons for implant removal were: procedure-related infection in 2 cases; lack of scaffold integration with scaffold loosening 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).

The analysis of the features of the failed patients (6 male, 2 female) revealed no difference in terms of age, sex, BMI, previous surgery, lesion size and lesion location compared to the non-failed group. In all failed cases, OA was graded as KL=2. In 5/8 cases, failures involved implants in the MFC, but this reflects the fact that the majority of patients of the present series received scaffold implantation in that location. In 7/8 patients a single site (MFC or LFC or throclea) was treated.

Histology
The specimen explanted from the patient who underwent TKR was sent to an independent lab (NAMSA, France) for GCP histologic 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 osteo-conduction and osteo-transduction, and formation of a well defined tidemark (Figure 5).

**Figure 5:** 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 2). 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<000.1 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.

In this complex scenario, JSLs treatment is considered a salvage option in order to avoid more aggressive procedures: in current practice both debridement and microfractures have been attempted, both being easy, unexpensive and largely available options. Unfortunately, none of them proved to be a reliable approach: high level evidence proved that arthroscopic lavage and debridement is not superior to physical and medical therapy alone,21 and, as shown by another RCT,36 this treatment couldn’t even provide superior benefit compared to sham surgery; these
findings suggests the lack of any influence on the overall course of disease. Also, microfractures, still considered the treatment of choice for chondral defects, showed limited benefit in large osteochondral lesions [24] with inconsistent results in OA patients,44,47 where the subchondral tissue is often metabolically impaired, and declining outcomes already at middle term evaluations.37 Repair tissue following microfractures is mainly fibrocartilaginous with weaker biomechanical properties, and some authors even suggested that failure of microfractures compromise the outcomes of cartilage revision surgery30 and, in case of concurrent OA, subchondral bone violation could conversely accelerate disease progression.3 In light of these results, new techniques have been proposed to promote regeneration of hyaline-like cartilage: anyway, even strategies such as MACT are influenced by the osteoarthritic environment, characterized by high concentrations of pro-inflammatory cytokines, metalloproteinases and other catabolic agents.10,14 OA has been considered a possible contra-indication for the use of MACT,16 but, when employed as a salvage procedure, it has showed some encouraging outcomes at least at short term: Minas et al.35 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. Ahlback grade 0-1). Kreutz et al.29 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 significant number of patients were treated with concurrent osteotomy, which may have played itself a significant role in the clinical outcome. On the other hand, when considering long term data, disappointing outcomes were recently published by Andriolo et al.2, 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.2 In fact, 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 knees33 and, secondly, the
impairment in subchondral bone 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. Therefore even MACT seems unable to influence the course of OA, and its use in
degenerative knees should be very cautious, with patients’ counselling fundamental to avoid
unrealistic expectations.

To overcome this drawback, and also other flaws 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 practices 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 cylindrical implant made of a polylactide-coglycolide copolymer, for which controversial
results were shown. Among the few case series published, Dhillander 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.

The findings of the present study support the regenerative potential of the aragonite scaffold 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.

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. Furthermore, 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. Although the low number of KL grade 3 patients treated, and the lower defect fill observed in MRI, clinical scores markedly improved and no failures occurred in this subgroup in the short term evaluation. These findings could be relevant since they might further support the role of biologic procedures as a “joint preservation” approach for patients not ready to receive metal resurfacing. Future research should confirm these data on larger cohort of patients, and also try to understand patient-related prognostic factors in order to optimize the clinical indications and select those 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 available 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. 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 whether it has the potential to delay joint replacement and be considered as a disease modifying treatment.

REFERENCES


38. OARSI White Paper- OA as a Serious Disease


**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 2:** 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 3:** 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 4:** 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 5: 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.