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

Results of a 2-Year Multicenter Prospective Study

Elizaveta Kon,[*†] MD, Berardo Di Matteo,[*†‡§] MD, PhD, Peter Verdonk,[‖¶] MD, Matej Drobnic,[#] MD, Oliver Dulic,[**] MD, Gordan Gavrilovic,[††] MD, Jenel M. Patrascu,[‡‡] MD, Ken Zaslav,[§§] MD, Grzegorz Kwiatkowski,[‖‖] MD, Nir Altschuler,[¶¶] MSc, and Dror Robinson,[##] MD

Investigation performed at the Rizzoli Orthopaedic Institute, Bologna, Italy; Ljubljana University Medical Centre, Ljubljana, Slovenia; Specialist Hospital Louis Rydygier, Krakow, Poland; AZ Monica Antwerpen, Antwerp, Belgium; Timişoara County Hospital, Timişoara, Romania; Institute for Orthopaedic Surgery ''Banjica,'' Belgrade, Serbia; Hasharon Hospital, Petah Tikva, Israel; and Humanitas Clinical and Research Center, Milan, Italy

Background: Osteoarthritis (OA) is considered a contraindication 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 joint surface lesions (JSLs) in OA knees.

Purpose: To present the 2-year follow-up clinical and radiological outcomes after implantation of a novel, cell-free aragonite-based 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: A total of 86 patients, 60 male and 26 female, with a mean age of 37.4 ± 10.0 years, mild to moderate knee OA, and a mean defect size of 3.0 ± 1.7 cm^2, were recruited at 8 medical centers according to the following criteria: radiographic mild to moderate knee OA (Kellgren-Lawrence grade 2 or 3); up to 3 treatable chondral/osteochondral defects (International Cartilage Repair Society grades 3 and 4) on the femoral condyles or trochlea; a total defect size $\leq$7 cm^2; and no concurrent knee instability, severe axial malalignment, or systemic arthropathy. All patients were evaluated at baseline and at 6, 12, 18, and 24 months after implantation using the Knee injury and Osteoarthritis Outcome Score (KOOS) and International Knee Documentation Committee (IKDC) subjective score. Additionally, magnetic resonance imaging (MRI) was performed to assess the amount of cartilage defect filling at the repaired site.

Results: Significant improvement on all KOOS subscales was recorded from baseline (Pain: 49.6 ± 13.1; Activities of Daily Living [ADL]: 56.1 ± 18.4; Sport: 22.8 ± 18.8; Quality of Life [QoL]: 23.5 ± 16.5; Symptoms: 55.4 ± 19.9) to the 24 months' follow-up (Pain: 79.5 ± 21.1 [$P < .001$]; ADL: 84.1 ± 21.4 [$P < .001$]; Sport: 60.8 ± 31.9 [$P < .001$]; QoL: 54.9 ± 30.4 [$P < .001$]; Symptoms: 77.7 ± 21.2 [$P < .001$]). The IKDC subjective score showed a similar trend and improved from 37.8 ± 14.7 at baseline to 65.8 ± 23.5 at 24 months ($P < .001$). MRI 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 osteochondral scaffold in patients with JSLs and mild to moderate knee OA provided significant clinical improvement at the 24-month follow-up, as reported by the patients. These findings were associated with good cartilage defect filling, as observed on MRI.

Keywords: osteoarthritis; aragonite; scaffold; cartilage regeneration; cartilage repair; osteochondral; Agili-C; joint preservation; cartiheal

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Knee joint surface lesions (JSLs), that is, chondral and osteochondral defects, have always been a treatment challenge for surgeons,[11] especially in the presence of joint degeneration. Osteoarthritis (OA) has been considered

a contraindication to cartilage repair procedures[16] because of a hostile joint environment in which the increased concentration of proinflammatory molecules and catabolic agents may impair potential cartilage healing.[31,41] 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 a serious disease with an unmet medical need,[4,18,38] and it cannot be considered a disease of the "elderly population" anymore.[31,38] In fact, in current practice, it has become common to diagnose JSLs in relatively young or middle-aged patients already presenting signs of mild to moderate OA, and the presence of these cartilage defects has been associated with disease severity and considered a predictor of joint replacement in the midterm.[46]

In the past 20 years, researchers have attempted to treat JSLs associated with mild or moderate OA with different strategies, from simple arthroscopic debridement[21,36] to microfracturing,[30,37,44] resulting in unsatisfactory outcomes in the majority of cases because of the impossibility of these treatment methods to modify the course of the disease. In more recent years, there have been also a few studies investigating matrix-assisted autologous chondrocyte transplantation (MACT) to treat medium to large focal defects in OA joints.[17,29,35] Results reported in the short term were fair,[29,35] but gradual worsening and high failure rates were observed after a longer evaluation period.[2] Regardless of the outcomes, the use of MACT has some significant drawbacks, which are mainly related to the 2-step surgical approach, resulting 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 subchondral bone abnormalities, which are inherent to OA, limits the use of this procedure.[19]

For this reason, cell-free scaffolds have been developed to promote the regeneration of both the subchondral bone and overlying cartilage in medium to large JSLs. Such 3-dimensional scaffolds have the advantage of being an off-the-shelf product, thus always available for use in the operating room. As such, they can be used to treat a JSL in a single-step surgical procedure.[16] Despite intense research in the fields of biomaterials and OA, only a few osteochondral scaffolds have reached clinical use. There are a lack of data on their performance, especially when used to treat JSLs in the OA environment.[7,13]

The scaffold tested in the present trial is a biphasic implant composed of inorganic calcium carbonate, that is, aragonite, which is a natural biomaterial with a 3-dimensional microarchitecture similar to human bone, including a comparable interconnected pore network[9] and a crystalline form of calcium carbonate ($CaCO_3$) analogous to physiological hydroxyapatite. Aragonite is derived from the coral exoskeleton, and its application in orthopaedics as a bone substitute is well-documented.[9] Although no preclinical study has compared aragonite with other similar osteochondral scaffolds, preclinical results were considered encouraging to adopt it in clinical practice. In fact, the unique feature of this novel scaffold is its ability to restore the subchondral bone, as documented by extensive in vitro studies, which showed not only its osteoinductive and osteoconductive capabilities but also unique osteotransductive properties, that is, the formation of bone through the direct deposition of bone trabeculae on the scaffold material.[1,15,20,34,43] The chondrogenic potential of the articular phase of the scaffold has been studied in another ex vivo trial,[5] and these findings were also confirmed in a goat model in which the scaffold was able to restore the entire osteochondral unit even in extremely large defects.[26-28]

The aim of this multicenter prospective study was to present the 2-year follow-up clinical and magnetic resonance imaging (MRI) outcomes after the implantation of a novel, cell-free aragonite-based scaffold in patients with chondral/osteochondral defects in the context of mild to

§Address correspondence to Berardo Di Matteo, MD, PhD, Humanitas Clinical and Research Center, Via Alessandro Manzoni 113, 20089 Rozzano, Milan, 20136, Italy (email: berardo.dimatteo@gmail.com).
*Department of Biomedical Sciences, Humanitas University, Milan, Italy.
†Humanitas Clinical and Research Center, IRCCS, Milan, Italy.
‡First Moscow State Medical University, Sechenov University, Moscow, Russia.
‖ORTHOCA, AZ Monica, Antwerp, Belgium.
¶Department of Orthopaedic Surgery, Antwerp University Hospital, Antwerp, Belgium.
#Department of Orthopedic Surgery, Ljubljana University Medical Centre, Ljubljana, Slovenia.
**Department of Orthopedic Surgery and Traumatology, Clinical Center of Vojvodina, Novi Sad, Serbia.
††Institute for Orthopaedic Surgery ''Banjica,'' Belgrade, Serbia.
‡‡Spitalul Clinic Judeţean de Urgenţă ''Pius Brînzeu'' Timişoara, Timişoara, Romania.
§§OrthoVirginia, Virginia Commonwealth University, Richmond, Virginia, USA.
‖‖Department of Knee Surgery, Arthroscopy and Sports Trauma, District Hospital of Orthopedics and Trauma Surgery, Piekary Slaskie, Poland.
¶¶CartiHeal Ltd, Kfar Saba, Israel.
##Orthopedic Research Unit and Foot and Ankle Service, Hasharon Hospital, Rabin Medical Center, Petah Tikva, Israel.
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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.

METHODS

Ethical Approval

The present multicenter prospective clinical study was approved by the hospital ethics committee and/or internal review board of each involved medical center and the regional ethics committee of Emilia-Romagna, Italy. Informed consent was obtained from all participating patients.

Patient Selection

Each participating site (8 European hospitals) is a recognized cartilage disease treatment referral center. Patient enrollment took place in 2016-2017, and before signing the consent form for study participation, all the patients were informed on the presence of degenerative changes in their knee and inherent implications. All patients were counseled about possible alternative treatments, and patients with Kellgren-Lawrence grade 3 OA were informed specifically about the option of arthroplasty. With regard to patient selection, the following criteria were used.

Inclusion criteria consisted of patients with the following: (1) age $\geq$18 years with mild to moderate OA according to radiographs (Kellgren-Lawrence grade 2 or 3) at baseline, (2) up to 3 treatable JSLs (chondral/osteochondral; International Cartilage Repair Society [ICRS] grade 3-4) located on the femoral condyles and/or trochlea, (3) a total treatable area ranging from 1 to 7 cm^2, and (4) Knee injury and Osteoarthritis Outcome Score (KOOS) Pain value at screening between 30 and 65. The choice of the aforementioned KOOS Pain value interval was made to avoid the inclusion of patients with symptoms too mild to justify surgical treatment at baseline (KOOS Pain >65) or patients with very intense pain (KOOS Pain <30) attributed mainly to the underlying OA who were considered to have unrealistic chances of a satisfactory outcome from the regenerative treatment proposed. Similar criteria have been used in other studies.[23,24]

Exclusion criteria consisted of patients with the following: (1) a bony defect depth deeper than 8 mm (based on preoperative imaging and intraoperative findings); (2) articular cartilage lesions in the tibia or patella with ICRS grade $\geq$3; (3) previous surgery in the index knee within the past 12 months; (4) presence of ligamentous instability; (5) lack of a functional remaining meniscus at the end of the procedure (ie, subtotal or total meniscectomy; concomitant partial meniscectomy was allowed); (6) untreated malalignment in the index knee (>5° varus or >5° valgus); (7) any known history of tumors, infections, 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 (BMI) >35; and (10) a history of any significant systemic disease such as, but not limited to, HIV, hepatitis, or HTLV infections and known coagulopathies that might compromise the patient's welfare.

Figure 1. The Agili-C aragonite-based scaffold. The microdrilled layer represents the surface of the scaffold, which should be placed 2 mm below the adjacent surrounding cartilage in a press-fit manner.

Scaffold Characteristics

The Agili-C scaffold (CartiHeal) is a porous, interconnected calcium carbonate (aragonite) implant derived from a purified, inorganic coral exoskeleton. The scaffold is biphasic: the lower part of the implant (subchondral phase) is composed of inorganic aragonite, characterized by a 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 microdrilled channels (Figure 1). This design promotes bone marrow and synovial mesenchymal stem cell 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.[5] The implants are sterilized by gamma irradiation, are 10 mm in height, and are available in a range of diameters to properly match the lesion size; those used in this study ranged from 10 to 17.5 mm in diameter.

Surgical Technique

The surgical technique was carried out while the patient was in a supine position under general or spinal anesthesia. A pneumatic tourniquet was applied to the proximal

thigh. Initially, standard knee arthroscopic surgery was performed to verify patient eligibility and to treat concurrent abnormalities (eg, meniscal tears, loose bodies) when necessary. Depending on the size and location of the defect(s), mini-arthrotomy was performed using a medial or lateral parapatellar approach to expose the lesions. The implantation site was prepared using a proprietary surgical toolset (CartiHeal); a perpendicular aligner was positioned in the lesion center to verify perpendicularity to the articular surface. The aligner was used to place a K-wire in the defect, as the surgical instruments were cannulated and threaded 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 was prepared. Next, a reamer was inserted to ensure that the correct depth was obtained, and finally, a shaper was introduced to achieve precise implant wall inclination. A 12 mm–deep cavity with perpendicular shoulders was thus created to allow press-fit fixation of the implant. The shaper and K-wire were removed, and the cavity was washed out with saline solution to eliminate debris. Peripheral cartilage remnants were trimmed using a proprietary cartilage cutter or surgical scalpel to ensure smooth edges and avoid invagination during implant insertion. The Agili-C implant was manually inserted into the prepared site: initially, it was firmly pushed with the thumb and, subsequently, gently inserted (without impaction to avoid implant breakage) using a silicone-covered tamper to its final position 2 mm below the adjacent articular cartilage. Testing on an animal model[26] has shown that placing the implant slightly below the articular surface increases the overall stability of the scaffold, which is firmly embedded in the cancellous bone, and also enhances cartilage healing because of the reduction of shear stresses exerted at the surface of the scaffold (Figure 2).

When multiple Agili-C implants were used, it was important to keep a bone bridge of at least 5 mm between implants to avoid impingement. Implant stability was tested by cyclic bending of the knee while the implant was under direct vision, both before and after tourniquet removal.

Postoperative Rehabilitation Protocol

The rehabilitation program was implemented based on our previous experience using another osteochondral scaffold[24,39,40] and included toe-touch weightbearing using crutches for 4 weeks and then increasing partial weightbearing to reach full weightbearing after 6 weeks. During the first 48 hours, cryotherapy in combination with a continuous passive motion device was applied and continued for 3 weeks, together with active-assisted range of motion exercises. Quadriceps isometric sets and electrostimulation were started immediately after surgery. Hydrotherapy was allowed after suture removal, and stationary cycling was introduced at 4 weeks when knee flexion reached about 100°.[22]

After the patient regained full active range of motion (approximately 3 months after surgery), proprioceptive/balance activities and unrestricted walking were allowed. Resistance muscle strengthening exercises started after 3 months, coupled with more demanding open kinetic chain (terminal leg extension) and closed kinetic chain (inner range quadriceps and modified leg press) exercises. Outdoor cycling was allowed 6 months after surgery; 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 1 year.

Clinical Evaluation

All patients were evaluated before the surgical procedure and during follow-up visits at 6, 12, 18, and 24 months. During these visits, they were clinically examined and questioned to assess their symptoms, physical status, and knee function using the KOOS and International Knee Documentation Committee (IKDC) subjective score.[6]

The primary endpoint of the study was the change in the KOOS value from baseline to 24 months' follow-up. Failure was defined as scaffold removal for any reason during the follow-up period. Any other re-intervention on the index knee was considered an "adverse event" and has been described in the dedicated paragraph in the Results section.

MRI Evaluation

All patients underwent 1.5-T or 3.0-T MRI at 6, 12, 18, and 24 months' follow-up. The following protocol was adopted: field of view, 14 cm; slice thickness, 3-3.5 mm; matrix, 512 $\times$ 256 (or 384); and receiver bandwidth, 80-120 Hx/pixel. Sequences included the following: (1) coronal intermediate-weighted (IW) fast spin echo (FSE) with no fat saturation (repetition time [TR] $\geq$ 3000 ms; echo time [TE] = 30-40 ms), (2) coronal proton density–weighted (PDW) FSE with fat saturation (TR $\geq$ 3000 ms; TE = 10-20 ms), (3) sagittal IW FSE with no fat saturation (TR $\geq$ 3000 ms; TE = 30-40 ms), (4) sagittal PDW FSE with fat saturation (TR $\geq$ 3000 ms; TE = 10-20 ms), (5) axial IW FSE with no fat saturation (TR $\geq$ 3000 ms; TE = 30-40 ms), (6) axial T2-weighted FSE with fat saturation (TR $\geq$ 3000 ms; TE $\geq$ 70 ms), (7) sagittal T1-weighted with no fat saturation (TR = 600-800 ms; TE = 10-20 ms), and (8) oblique PDW FSE with fat saturation (TR $\geq$ 3000 ms; TE = 10-20 ms) oriented perpendicularly to the scaffold.

A defect fill repair assessment (0%-100%) was performed in a blinded manner by an independent radiologist, who is an expert in cartilage repair assessments. 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 sequence, 2 to 3 slices located within the implant were assessed: for each slice, the degree of cartilage defect volume fill was semiquantitatively assessed in increments of 25% (ie, 0%-24% fill, 25%-49% fill, 50%-74% fill, and 75%-100% fill). 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.

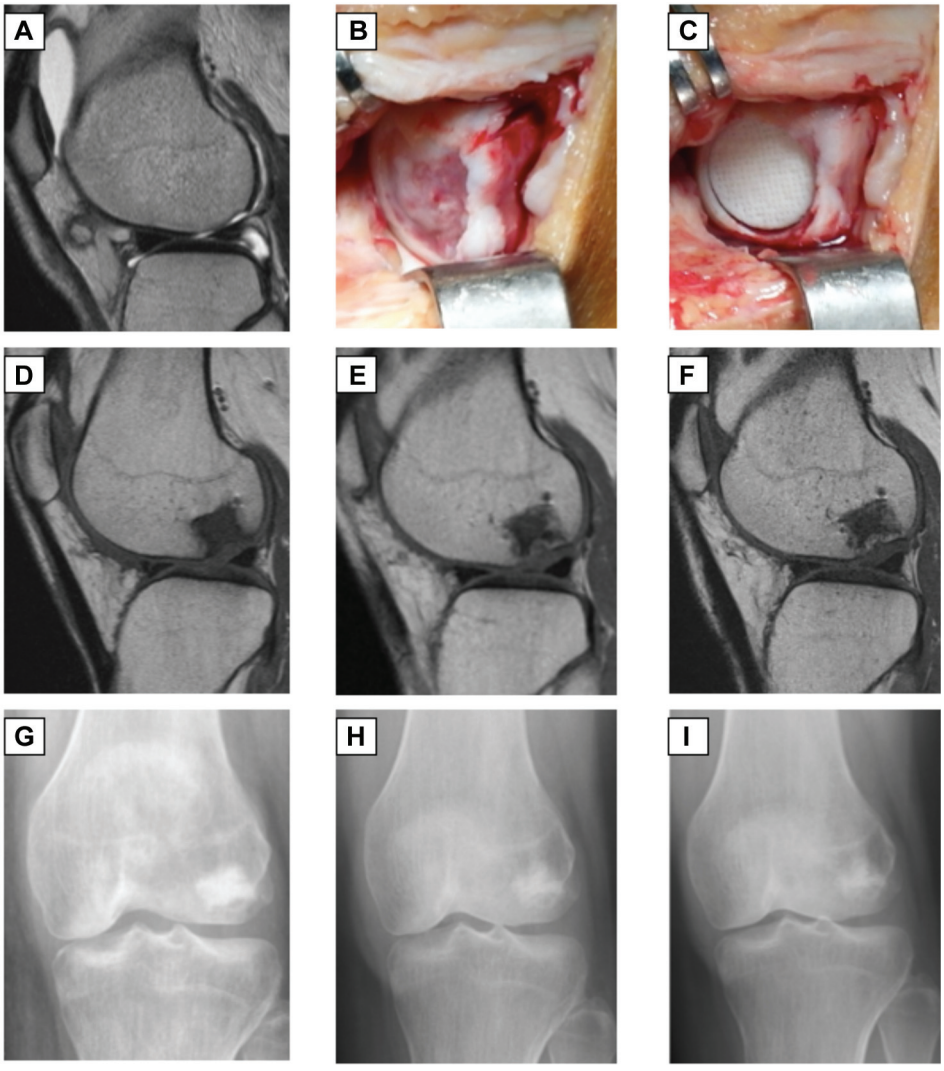

Figure 2. A 32-year-old woman with mild osteoarthritis and a large osteochondral defect on her lateral femoral condyle. The patient was treated with a single aragonite-based implant. (A) Magnetic resonance imaging (MRI) at baseline. (B) Intraoperative view of the defect. (C) Agili-C implantation (note the position 2 mm below the articular surface). (D) MRI at 6 months. (E) MRI at 12 months. (F) MRI at 24 months. (G) Radiograph at 6 months. (H) Radiograph at 12 months. (I) Radiograph at 24 months. Overall Knee injury and Osteoarthritis Outcome Score value improvement: 29.1 at baseline, 50.3 at 6 months, 72.9 at 12 months, 76.8 at 18 months, and 93.6 at 24 months.

Histological Evaluation

Evaluation was performed in one patient who subsequently underwent total knee replacement. Upon receipt of the harvested condyle, the specimen was cut using a microcutting technique (Exakt) to isolate the implanted site. One portion was dehydrated in alcohol solution, cleared in xylene, and embedded in a polymethyl methacrylate resin block, which was then cut longitudinally to obtain 3 sections for Paragon staining.

The other portion of the specimen was rinsed and decalcified in EDTA solution. After complete decalcification and dehydration in alcohol solution of increasing concentrations, the specimens were cleared in xylene and embedded in paraffin. The embedded specimens were then longitudinally cut (5-μm thickness ± 0.5 μm) using a microtome (Microm Microtech). A total of 5 central full-length serial sections per block were prepared and stained with modified Masson trichrome, safranin O/hematoxylin and eosin, and safranin O/Fast Green. Moreover, 2 sections were used for immunohistochemical determination of the presence of collagen type I and collagen type II.

Statistical Analysis

All continuous data were expressed as the mean and SD; categorical variables were expressed as the frequency and percentage. Differences among time points were explored with repeated-measures analyses of variance

and mixed-effects models. Multiple comparison P values were Bonferroni corrected.

For all tests, $P < .05$ was considered significant. All statistical analyses were performed with SPSS Version 19.0 (IBM).

RESULTS

Patient Characteristics

A total of 86 patients, 60 men and 26 women, were treated in this study. The mean age was 37.4 ± 10.0 years, the mean BMI was 26.1 ± 3.5, and the lesion size averaged 3.0 ± 1.7 cm^2. Patient data are summarized in Table 1. Ultimately, 6 of 86 patients were lost to follow-up at the 24-month follow-up.

Clinical Scores

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

KOOS subscores showed a significant increase from baseline to the 6-month follow-up ($P < .001$ in all cases) (all values reported in Table 2), with further improvements at 12-, 18-, and 24-month follow-up (Table 2 and Figure 3). The IKDC subjective score showed a similar trend, with a significant increase from baseline to the 6-month follow-up (37.8 ± 14.7 vs 55.4 ± 21.5, respectively; $P < .001$), followed by further significant improvements at 12, 18, and 24 months (Figure 4).

A subgroup analysis was performed comparing the clinical outcomes of patients with Kellgren-Lawrence grade 2 versus grade 3 OA, and no significant difference was observed in the clinical scores at any follow-up visit.

Adverse Events and Failures

Overall, 36 patients (including those defined as failures) experienced adverse events during the study duration:

- 15 patients experienced episodes of knee swelling and pain and were treated nonoperatively by a decrease in physical therapy/working activities, local cryotherapy, and oral nonsteroidal anti-inflammatory drugs;
- 3 patients presented with knee stiffness: 2 patients required knee manipulation under anesthesia, whereas 1 patient required prolonged rehabilitation;
- 1 patient had delayed surgical wound healing, managed by serial dressings that allowed complete healing of the wound;
- 3 patients complained about the onset of knee pain that was attributed to overloading during physical therapy; all were managed by adapting the rehabilitation program, and a resolution of pain was achieved;
- 2 patients had knee trauma during follow-up: in 1 case, a medial meniscal tear occurred, and the patient underwent partial meniscectomy, whereas in the other case, a hyperextension trauma caused low-grade muscle strain that was managed nonoperatively;

TABLE 1
Patient Characteristics[a]

	Value (N = 86)
Age, mean ± SD, y	37.4 ± 10.0
BMI, mean ± SD	26.1 ± 3.5
Sex	
Male	60 (69.8)
Female	26 (30.2)
Previous surgery in affected knee	48 (55.8)
ICRS grade	
Grade 3	21 (24.4)
Grade 4	65 (75.6)
Lesion size, mean ± SD, cm^2	3.0 ± 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)
Kellgren-Lawrence grade	
Grade 2	75 (87.2)
Grade 3	11 (12.8)
Concomitant procedures	19 (22.1)
High tibial osteotomy, n	2
Partial meniscectomy, n	8
Meniscal suturing, n	1
Debridement of other superficial lesions (ICRS grade 1 or 2), n	4
Loose body removal, n	3
Synovial plica removal, n	1

[a]Data are expressed as n (%) unless otherwise indicated. BMI, body mass index; ICRS, International Cartilage Repair Society.

- 1 patient underwent loose body removal (bony fragment) that caused occasional locking episodes;
- 1 patient suffered from patellar tendinitis that was managed nonoperatively;
- 1 patient complained about persisting quadriceps weakness; and
- 1 patient showed synovial hypertrophy and exuberant intra-articular scar tissue and underwent arthroscopic synovectomy and scar tissue removal; the site of scaffold implantation was inspected without detecting any problem.

There were 8 patients (9.3%) who underwent implant removal during the 2-year follow-up and were considered failures. In all cases, a relapse of severe knee pain with swelling and consequent motion limitation was observed. Reasons for implant removal were a procedure-related infection in 2 cases, lack of scaffold integration with scaffold partial loosening in 5 cases, and progression of OA in the patellofemoral compartment in 1 case (this patient underwent total knee replacement at 14 months after scaffold implantation on the medial femoral condyle).

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, or lesion location compared with the nonfailed patients. In all failed cases, OA was graded as Kellgren-Lawrence grade 2. In 5 of 8 cases, failures involved implants in the medial femoral condyle, but this reflects the fact that the majority of patients

TABLE 2
Clinical Scores and MRI Findings[a]

	Baseline	6 mo	12 mo	18 mo	24 mo	*P* Value (24 mo vs Baseline)
KOOS Pain	49.6 ± 13.1	73.0 ± 21.1[b]	77.5 ± 19.6	78.1 ± 21.1	79.5 ± 21.1[c]	<.001
KOOS ADL	56.1 ± 18.4	78.7 ± 20.9[b]	82.5 ± 18.9	83.5 ± 20.3	84.1 ± 21.4	<.001
KOOS Sport	22.8 ± 18.8	48.1 ± 29.5[b]	55.5 ± 29.9	56.0 ± 31.9	60.8 ± 31.9[c]	<.001
KOOS Symptoms	55.4 ± 19.9	71.9 ± 21.7[b]	75.9 ± 19.8	76.1 ± 22.0	77.7 ± 21.2	<.001
KOOS QoL	23.5 ± 16.5	44.7 ± 27.6[b]	48.7 ± 26.3	52.4 ± 27.7	54.9 ± 30.4[c]	<.001
KOOS overall	41.5 ± 14.3	63.3 ± 21.7[b]	68.0 ± 20.9	69.2 ± 22.8	71.4 ± 23.6[c]	<.001
IKDC	37.8 ± 14.7	55.4 ± 21.5[b]	62.2 ± 20.6[d]	63.6 ± 21.6	65.8 ± 23.5	<.001
MRI defect fill, %	N/A	63.7 ± 29.1	70.3 ± 28.6	77.7 ± 26.0	78.7 ± 25.3[c]	N/A

[a]Data are expressed as mean ± SD. ADL, Activities of Daily Living; IKDC, International Knee Documentation Committee; KOOS, Knee injury and Osteoarthritis Outcome Score; MRI, magnetic resonance imaging; N/A, not applicable; QoL, Quality of Life.
[b]Significant difference compared with baseline ($P < .001$).
[c]Significant difference compared with 6 months ($P < .001$).
[d]Significant difference compared with 6 months ($P = .01$).

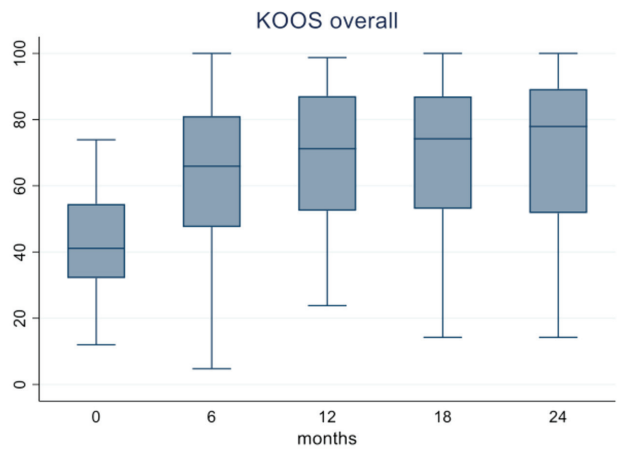

Figure 3. Overall Knee injury and Osteoarthritis Outcome Score (KOOS) value trend at baseline and 6-, 12-, 18-, and 24-month follow-up (box-and-whisker plots showing median, interquartile range, and range).

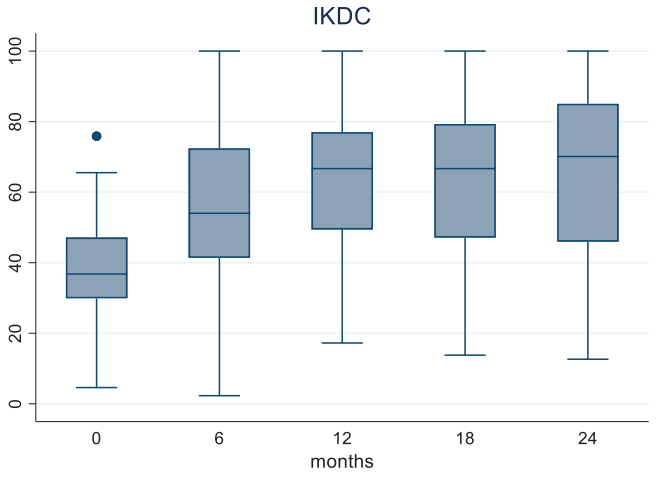

Figure 4. International Knee Documentation Committee (IKDC) subjective score trend at baseline and 6-, 12-, 18-, and 24-month follow-up (box-and-whisker plots showing median, interquartile range, and range).

in the present series underwent scaffold implantation in that location. In 7 of 8 patients, a single site (medial or lateral femoral condyle or trochlea) was treated.

Histology

The specimen explanted from the patient who underwent total knee replacement was sent to an independent laboratory (NAMSA) for good laboratory practice (GLP) histological analysis, which revealed (1) the new 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 homogeneous proteoglycan expression; and (2) the restoration of the subchondral bone plate with trabecular architecture and integration within the surrounding native bone through osteoconduction, osteotransduction, and the formation of a well-defined tidemark (Figure 5).

MRI Evaluation

All patients except those lost to follow-up and treatment failures completed the 24-month follow-up. A significant increase in the area of the defect covered by cartilage regrowth was observed (Figure 2). As early as 6 months after implantation, significant defect fill was observed (63.7 ± 29.1); the degree of defect fill continued to improve at the 12 and 18 month follow-ups (70.3 ± 28.6 and 77.7 ± 26.0, respectively) and reached a maximum after 24 months (78.7 ± 25.3; $P < .001$ vs 6 months' value) (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)

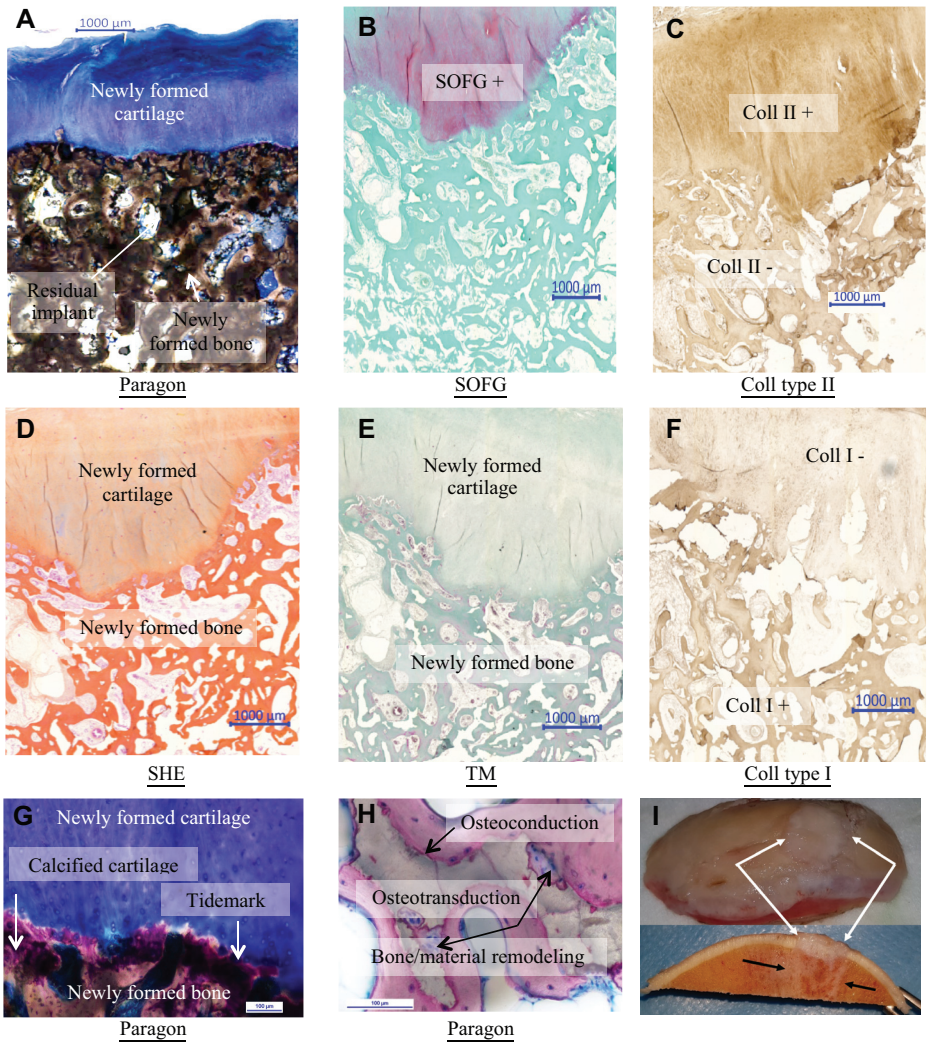

Figure 5. Histological evaluation of the explanted specimen. (A) Paragon staining indicating the regeneration of new articular cartilage and subchondral bone through implant remodeling. (B) Safranin O/Fast Green (SOFG) staining indicating a high level of proteoglycan content in the newly formed cartilage. (C) Collagen type II marker indicating hyaline cartilage formation. (D) Safranin O/hematoxylin and eosin staining indicating the absence of inflammatory reactions. (E) Masson trichrome staining indicating a general morphological assessment of repaired tissue. (F) Collagen type I marker indicating an absence of collagen type I in the cartilage and the presence of collagen type I in the repaired bone. (G) Paragon staining indicating a newly formed tidemark and calcified cartilage. (H) Paragon staining indicating osteoconduction and osteotransduction (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, and black arrows indicate implant/bone remodeling with good restoration of the subchondral bone tissue.

in knees with mild to moderate OA. The evidence of MRI defect fill at the scaffold's surface supports the positive clinical outcomes. 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, JSL treatment is considered a salvage option to avoid more aggressive procedures. In current practice, debridement and microfracture have been attempted, with both being easy, inexpensive, and largely available options. Unfortunately, none of them proved to be a reliable approach; high-level evidence proved that arthroscopic lavage and debridement are not superior to physical and medical therapies alone,[21] and as shown by another randomized controlled trial,[36] this treatment could not even provide superior benefit compared with sham surgery. These findings suggest the lack of any influence on the overall course of the disease. Also, microfracture, 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] in whom the subchondral tissue is often

metabolically impaired, and declining outcomes in midterm evaluations.[37] Repair tissue after microfracture is mainly fibrocartilaginous with weaker biomechanical properties, and some authors have even suggested that failure of microfracture compromises the outcomes of cartilage revision surgery[30]; and in the 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 the regeneration of the articular cartilage; even strategies such as MACT are influenced by the OA environment, characterized by high concentrations of proinflammatory cytokines, metalloproteinases, and other catabolic agents.[10,14] OA has been considered a possible contraindication for the use of MACT,[16] but when employed as a salvage procedure, it has shown some encouraging outcomes at least in the short term. Minas et al[35] documented significantly improved 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 (ie, Ahlback grade 0-1). Kreuz et al[29] treated a cohort of 19 patients who presented a more advanced level of OA (Kellgren-Lawrence grade 2 or 3) and revealed satisfactory outcomes in the midterm evaluation (4 years). Interestingly, in both studies, a significant number of patients were treated with concurrent osteotomy, which may have played an important role in the clinical outcomes. 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 biological properties as those taken from healthy knees,[33] and second, the impairment in subchondral bone may affect graft survival.[19] Biochemical and physical alterations in the subchondral bone region are always present in OA knees, and therefore, a "surface" treatment such as MACT can be negatively affected by these pathological changes in the long term. Therefore, even MACT seems unable to influence the course of OA, and its use in knees with degenerative disease should be very cautious, with patient counseling fundamental to avoid unrealistic expectations.

To overcome this drawback, and also other flaws such as the need for 2 surgical steps and cell manipulation,[25] cell-free scaffolds have been introduced.[16] These biomaterials have been developed with the aim of promoting tissue regeneration at the level of both the subchondral bone and the cartilage layer, without the need for cell expansion, by recruiting resident autologous mesenchymal stem cells. The mechanism of action consists of providing a microenvironment in which cells can differentiate and produce an extracellular matrix. The scaffolds have different layers that promote the concurrent restoration of subchondral bone and cartilage.[26,27] Despite extensive preclinical research in the field of biomaterials, only a few osteochondral scaffolds have reached clinical practice,[8,32,45] and in spite of promising results demonstrated in animal models, their regenerative potential in the human setting has thus far shown less favorable outcomes, especially concerning the subchondral bone. The first scaffold available was a bilayered cylindrical implant made of a PLGA copolymer, for which controversial results were demonstrated.[45] Among the few case series published, Dhollander et al[12] recorded a failure rate of 20% (3/15 patients) at 1-year follow-up, and biopsy specimens 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 collagen type I at different percentages within the various layers.[7] Essentially, 2 trials[7,42] investigated the use of this scaffold in patients with early OA. Condello et al[7] documented a success rate of only 69% in a cohort of 26 patients evaluated up to 3 years, whereas Sessa et al[42] 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 computed tomography showed slow and limited subchondral bone healing,[42] which could affect long-term outcomes, especially in patients with complex disease.

The findings of the present study support the regenerative potential of the aragonite-based scaffold in patients with complex knee injuries, such those affected by mild to moderate OA. MRI revealed good articuar cartilage regeneration and good subchondral bone reconstruction as the scaffold gradually degraded over time. These findings were also confirmed by a histological examination conducted on an explanted specimen: regeneration of the osteochondral unit was proven by the presence of newly formed hyaline cartilage, rich in collagen type II and proteoglycans and lacking in 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 tissue was well-integrated within the adjacent native cartilage and bone.

The results presented are particularly relevant for a number of reasons: first, because of 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 (N = 86). Another major point is that, as opposed to other reports,[7,42] only a small number of patients in the present series (n = 19) underwent concurrent surgery (only 2 were major procedures, ie, high tibial osteotomy), thus allowing a 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 Kellgren-Lawrence grade 3 OA, for whom there are minimal data in the published literature. Regardless of the low number of patients with Kellgren-Lawrence grade 3 OA treated and the lower defect fill observed on MRI, clinical scores markedly improved, and no failures occurred in this subgroup at the short-term follow-up. These findings could be relevant because they might further support the

role of biological procedures as a "joint preservation" approach for patients not ready to undergo metal resurfacing. Future research should confirm these data on a larger cohort of patients and also try to understand patient-related prognostic factors to optimize 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 histological data must be acknowledged, as only 1 full specimen was available and analyzed from a patient who underwent total knee replacement because of the progression of OA in the patellofemoral compartment.

The Agili-C implant for the treatment of ICRS grade 3 to 4 defects in OA knees provided promising clinical and radiological outcomes at 2 years, suggesting that the aragonite-based scaffold was capable of promoting good 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 comparing this implant technique with the surgical standard of care are required to assess if this implant is a superior treatment option. Additionally, longer term evaluations are required to assess the durability of the outcomes to understand whether it has the potential to delay joint replacement and be considered a disease-modifying treatment method.

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