Aragonite-Based Scaffold Versus Microfracture and Debridement for the Treatment of Knee Chondral and Osteochondral Lesions

Results of a Multicenter Randomized Controlled Trial

Nir Altschuler,* MSc, Kenneth R. Zaslav,† MD, Berardo Di Matteo,‡§‖ MD, PhD, Seth L. Sherman,¶ MD, Andreas H. Gomoll,# MD, Scott A. Hacker,** MD, MS, Peter Verdonk,†† MD, PhD, Oliver Dulic,‡‡ MD, PhD, Jenel M. Patrascu,§§ MD, PhD, Andrew S. Levy,‖ ‖ MD, Dror Robinson,¶¶ MD, PhD, and Elizaveta Kon,‡§ MD
Investigation performed at the IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy

Background: Lesions of the articular cartilage, with or without involvement of the subchondral bone, are a common cause of pain and dysfunction in the knee. Although several treatment options have been developed, the majority of previous clinical trials examined patients with isolated or focal midsized defects, which rarely represent the condition found in the general population. Rather, cartilage lesions are often associated with the presence of mild to moderate osteoarthritic changes.

Purpose: The present multicenter randomized controlled trial compared the clinical and radiographic outcomes of an aragonite-based osteochondral implant with a control group (arthroscopic debridement/microfractures) in patients affected by joint surface lesions of the knee, including those with concurrent mild to moderate osteoarthritis.

Study Design: Randomized controlled trial; Level of evidence, 1.

Methods: A total of 251 patients were enrolled in 26 medical centers according to the following criteria: age 21 to 75 years, up to 3 cartilage defects of International Cartilage Regeneration & Joint Preservation Society grade 3a or above located on the femoral condyles and/or trochlea, total treatable area from 1 to 7 cm^2, bony defect depth $\leq$8 mm, and knee osteoarthritis grade 0 to 3 according to Kellgren-Lawrence score. Patients were randomized to the aragonite-based implant or debridement/microfracture control arm in a 2:1 ratio. Evaluation was performed at 6, 12, 18, and 24 months based on overall Knee injury and Osteoarthritis Outcome Score (KOOS) as the primary endpoint, and the KOOS subscales (Pain, Quality of Life, Activities of Daily Living), percentage of responders, and International Knee Documentation Committee (IKDC) subjective score as the secondary endpoints. Patients also underwent magnetic resonance imaging evaluation at 12 and 24 months to assess defect fill grade. Failures (ie, need for any secondary treatment) and adverse events were also recorded.

Results: The implant group showed a statistically superior outcome in the primary endpoint and all secondary endpoints at each follow-up. The magnitude of improvement in the implant group was twice as large as that in the control group in terms of mean KOOS improvement at 2 years. Responder rate (defined as at least a 30-point improvement in overall KOOS) was 77.8% in the implant group as opposed to 33.6% in the control ($P < .0001$). Statistically superior results were seen in the IKDC score as well. At 24 months, 88.5% of the implanted group had at least 75% defect fill on magnetic resonance imaging as compared with 30.9% of controls ($P < .0001$). The failure rate was 7.2% for the implant group versus 21.4% for control.

Conclusion: This aragonite-based scaffold was safe and effective in the treatment of chondral and osteochondral lesions in the knee, including patients with mild to moderate osteoarthritis, and provided superior outcomes as compared with the control group.

Registration: NCT03299959 (ClinicalTrials.gov identifier).

Keywords: osteoarthritis; aragonite; scaffold; cartilage regeneration; osteochondral; microfracture

The American Journal of Sports Medicine
2023;51(4):957–967
DOI: 10.1177/03635465231151252

Knee articular cartilage lesions are common and often associated with a severe effect on patients' quality of life and working ability.[14,16,34] If left untreated, these lesions

can deteriorate into osteoarthritis (OA),[11] a condition that has been recognized as one of the leading causes of disability worldwide.[12,31] Chondral and osteochondral lesions are frequently seen in patients with OA; thus, there is an urgent need for novel treatment options to preserve these joints and delay the need for joint replacement.

Several treatment options have been developed to manage chondral and osteochondral lesions, primarily depending on the size and depth of the defects.[15] To date, no gold standard treatment has been defined for this unmet need. Although positive outcomes have been reported for many of these techniques, the majority of published clinical trials have examined patients with isolated or focal midsized defects, which rarely represent the clinical conditions found in the general patient population.[30,38] Specifically, chondral and osteochondral lesions are often associated with other joint comorbidities—most notably, the presence of meniscal tears, malalignment, and osteoarthritic changes. Several studies suggest that these comorbidities may have a negative effect on the outcomes of these procedures.[27,39]

Currently, the most common surgical standard of care (SSOC) used for cartilage lesions is arthroscopic debridement or microfracture, which includes removal of the unstable cartilage fragments (debridement) and deep penetration of the subchondral bone (microfracture) to stimulate bone marrow cellular components to repair the joint surface.[13,37] However, while the repair achieved through microfracture is effective at producing fibrocartilage, the regrown tissue is composed of fibrocartilage, not the hyaline cartilage originally present. Fibrocartilage deteriorates over time, with noticeable diminished clinical results after 4 years.[29,35] Over the past 20 years, innovations in the field of biotechnology have led to the introduction of novel techniques to address chondral/osteochondral defects, such as matrix-assisted autologous chondrocyte implantation (ACI) and various natural and manufactured scaffolds with the aim of regenerating hyaline articular cartilage.[1,22]

The current randomized multicenter pivotal trial is an investigational device exemption study that examined the potential superiority of a novel 3-dimensionally structured off-the-shelf scaffold (Agili-C; Cartiheal Ltd) over the SSOC in the treatment of knee chondral and osteochondral lesions. This scaffold is produced from a coralline exoskeleton in the crystalline form of aragonite, a natural biomaterial with a 3-dimensional microarchitecture very similar to human cancellous bone.[9,28] This biomaterial has revealed osteoinductive, osteoconductive, and osteotransductive capabilities,[23-25] as well as, when modified, chondrogenic potential.[22] The primary endpoint of the study was to compare the performance of the aragonite-based scaffold with that of SSOC (microfracture/debridement) at 24-month follow-up by evaluating clinical and radiographic results in a broad population of patients. This study is unique among trials treating these types of lesions, as it included patients affected by mild to moderate knee OA (Kellgren-Lawrence [KL] 2 or 3).

METHODS

Study Design and Randomization Process

The study enrolled patients at 26 medical centers. The protocol was approved by the Food and Drug Administration (FDA) as well as the relevant ethical committees/internal review boards of all sites. All patients signed an informed

‖Address correspondence to Berardo Di Matteo, MD, PhD, Department of Biomedical Sciences, Humanitas University, Via Rita Levi Montalcini 4, 20090 Pieve Emanuele (Milan), Italy (email: berardo.dimatteo@gmail.com).

*Cartiheal Ltd, Kfar Saba, Israel.

†Lennox Hill Hospital–Northwell Health Orthopedic Institute, New York, New York, USA.

‡IRCCS Humanitas Research Hospital, Rozzano, Milan, Italy.

§Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Milan, Italy.

¶Department of Orthopaedic Surgery, Stanford University, Stanford, California, USA.

#Hospital for Special Surgery–Orthopedic Surgery and Sports Medicine, New York, New York, USA.

**Grossmont Orthopedic Medical Group, San Diego, California, USA.

††Orthoca, Antwerp, Belgium.

‡‡Medical Faculty, University of Novi Sad; Department for Orthopedic Surgery and Traumatology, University Clinical Center of Vojvodina, Novi Sad, Serbia.

§§Victor Babeş Timisoara University of Medicine and Pharmacy; Timisoara, Romania.

‖‖Center for Advanced Sports Medicine, Knee and Shoulder, Millburn, New Jersey, USA.

¶¶Robinson Clinic, Bat Yam, Israel.

Submitted August 25, 2022; accepted December 13, 2022.

One or more of the authors has declared the following potential conflict of interest or source of funding: The present trial was fully funded by Cartiheal Ltd, Israel. N.A. has received stock options and consulting fees from Cartiheal Ltd. K.R.Z. has received consulting fees from Bioventus, Vericel, and Cartiheal Ltd; compensation for services other than consulting from Stryker; support for education from Arthrex and Supreme Orthopedic Systems; and hospitality payments from Linvatec. K.R.Z. also holds stock options in Cartiheal Ltd. S.L.S. has received consulting fees from Smith & Nephew, Vericel Corp, DJO LLC, Flexion Therapeutics Inc, Olympus American Inc, Bioventus LLC, Linvatec, Kinamed Inc, and Pacira; royalties from Linvatec; honoraria from Vericel Corp and Flexion Therapeutics; speaking fees from Arthrex Inc; and support for education from Elite Orthopedics and Evolution Surgical Inc. A.H.G. has received consulting fees from Smith & Nephew, JRF Ortho, Vericel, and Bioventus; speaking fees from Pacira Pharmaceuticals Inc and Linvatec; royalties from Organogenesis; and honoraria from Vericel, JRF Ortho, and Fidia Pharma. S.A.H. has received consulting fees from Heron Therapeutics, Anika Therapeutics, and Bioventus; honoraria from Encore Medical; and hospitality payments from Aesculap Biologics and Novartis Pharmaceuticals. P.V. has received consulting fees from and holds stock options in Cartiheal Ltd. A.L. has received consulting fees from Bioventus and Cartiheal Ltd; hospitality payments from Smith & Nephew and Encore Medical; and support for education from Zimmer Biomet. A.L. also holds stock options in Cartiheal Ltd. D.R. has received consulting fees from and holds stock options in Cartiheal Ltd. E.K. has received consulting fees from and holds stock options in Cartiheal Ltd. AOSSM checks author disclosures against the Open Payments Database (OPD). AOSSM has not conducted an independent investigation on the OPD and disclaims any liability or responsibility relating thereto.

consent form before study inclusion. Patients were randomized to either the aragonite-based scaffold or SSOC in a 2:1 ratio. Patients with focal defects and KL score 0 or 1 were randomized against similar patients treated with microfracture, while patients with mild to moderate OA (KL 2 or 3) were randomized against those treated with arthroscopic debridement.

Patients found eligible during the screening visit underwent arthroscopy to assess whether all the inclusion/exclusion criteria were met. All arthroscopic findings were recorded directly into a dedicated application in a tablet provided to each site, and the group assignment was provided in real time by the application so that the surgeon could proceed with the assigned treatment. Patient enrollment started in September 2017 and was completed in November 2019.

The clinical hypothesis underlying the present randomized controlled trial (RCT) was that the aragonite-based implant would be superior to the SSOC when measuring improvement in the overall Knee injury and Osteoarthritis Outcome Score (KOOS) at 24-month follow-up as compared with baseline.[6]

Study Population

Main inclusion criteria were as follows: patients aged 21 to 75 years, presence of up to 3 joint surface lesions International Cartilage Regeneration & Joint Preservation Society (ICRS) grade 3a or above on the femoral condyles or trochlea, total treatable area of 1 to 7 cm^2, patients physically and mentally willing and able to comply with the postoperative rehabilitation protocol and scheduled clinical and radiographic visits, and nonresponsive to physical therapy for at least 3 to 4 weeks.

The main exclusion criteria were as follows: (1) KOOS Pain subscale score at baseline <20 or >65 (maximum pain = 0, pain-free = 100); (2) bony defect depth >8 mm according to baseline magnetic resonance imaging (MRI), radiograph, or arthroscopy; (3) articular cartilage lesions in the tibia or the patella ICRS grade 4a or above; (4) severe OA of the index knee, graded 4 according to the KL score; (5) significant instability of the index knee according to International Knee Documentation Committee (IKDC) Knee Examination Form 2000, grade C (abnormal) or D (severely abnormal); (6) >8° varus or >8° valgus malalignment according to standing radiograph; (7) lack of functional remaining meniscus, ≥5-mm rim at the end of the procedure; (8) any known history of intra-articular or osseous infection of the index knee; (9) uncontained lesion—lack of vital bone wall ≥2 mm thick completely surrounding the lesion—based on MRI, radiograph, or arthroscopy; (10) inability to position the implant 2 mm recessed relative to the articular surface based on MRI, radiograph, or arthroscopy.

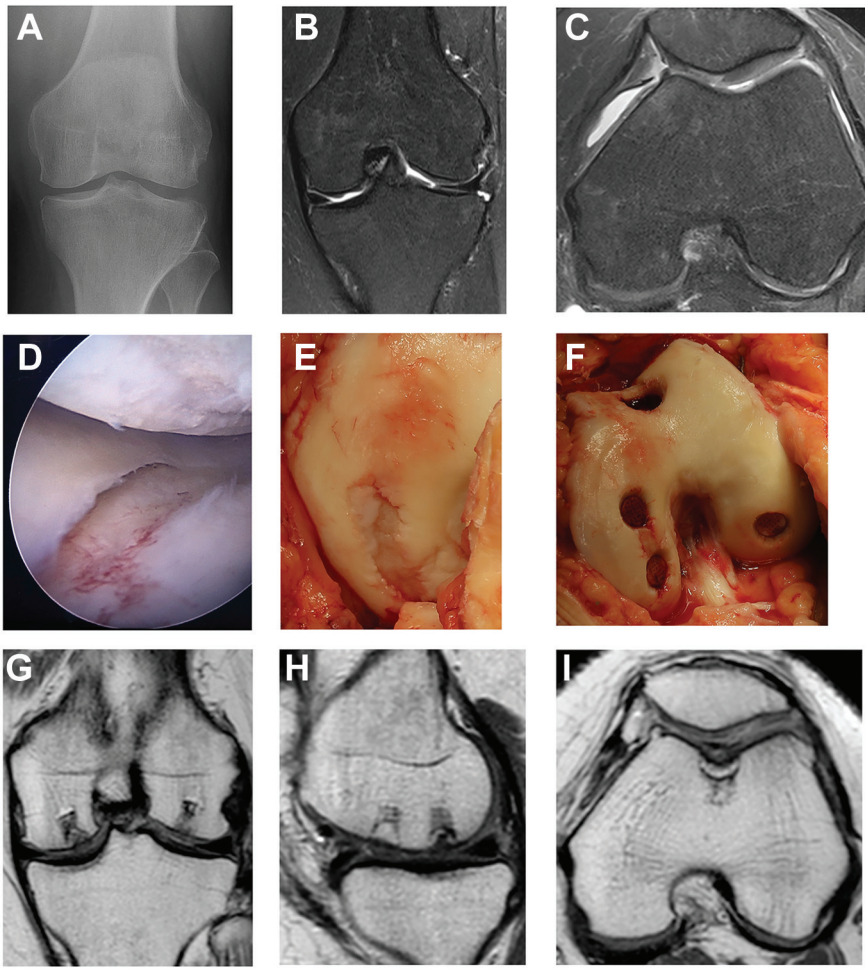
Study Procedures

Investigational Arm. Patients randomized to the investigational arm received the aragonite-based biphasic implant, which is cell-free, off-the-shelf, porous, biocompatible, and biodegradable. It consists of interconnected, natural, inorganic calcium carbonate (aragonite) derived from purified, inorganic coral exoskeleton. This material provides a 3-dimensional structure with the mechanical properties and high interconnected macroporosity required for vascular tissue ingrowth. Animal studies have shown that the bottom two-thirds of the implant encourages bone repair, whereas the top third, which has holes drilled in a specific diameter and pattern, attracts chondrocytes and stimulates cartilage repair.[20,24] The surgical technique has been described in previous studies[19,20]: briefly, the surgical site is prepared via a mini-open or open technique by sequential drilling through the articular surface into the subchondral bone using the dedicated instrument set. Once the preparation is complete, the implant is press-fit into the implantation site such that the top of the implant is positioned at the level of the subchondral bone ≥2 mm below the articular surface (Figure 1). When multiple implants are used, it is important to keep a bone bridge of ≥5 mm between implants to avoid implant impingement. Implant stability is tested by cyclic bending of the knee while the implant is under direct vision, before and after tourniquet removal. No major concurrent procedures (ie, osteotomies) were performed on any patient of the investigational arm.

Control Arm. Patients randomized to the SSOC arm were treated with arthroscopic debridement/microfractures. Debridement consisted of removing the damaged and unstable cartilage fragments from the articular surface. Microfracture consisted of penetrating the subchondral bone with a dedicated pick to stimulate bone marrow cellular components to restore the articular surface through formation of fibrocartilage.[29] No major concurrent procedures (ie, osteotomies) were performed on any patient of the control arm.

Rehabilitation Protocol

The recommended rehabilitation program included limited partial weightbearing (using crutches for 4 weeks), with 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, with active assisted range of motion exercises. Quadriceps isometric sets and electrostimulation were initiated immediately after surgery. Stationary cycling was introduced at 4 weeks, when knee flexion reached about 100°. Hydrotherapy was advised immediately after suture removal. After approximately 2 months, most patients were able to regain full active range of motion and could introduce proprioceptive/balance activities, walking, and resistance. Resistance muscle-strengthening exercises could be started after 3 months, coupled with a more demanding set of exercises: open kinetic chain (terminal leg extension) and closed kinetic chain (inner range quadriceps and modified leg press). Outdoor cycling activity and skiing were allowed 6 months after the operation. Repetitive joint impact activities were allowed after 1 year.



	Baseline	6M	12M	18M	24M
IKDC	19.54	62.07	62.07	88.51	90.80
KOOS Pain	25	80.56	75	91.67	94.44
KOOS QOL	12.5	68.75	68.75	93.75	100
KOOS Symptoms	42.86	78.57	75	89.29	92.86
KOOS ADL	17.65	88.24	75	100	100
KOOS Sport	0	60	55	100	100
KOOS Overall	**19.60**	**75.22**	**69.75**	**94.94**	**97.46**

Figure 1. Imaging and clinical scores for a female patient, age 58 years old, with Kellgren-Lawrence osteoarthritis grade 2. (A) Baseline radiograph. (B, C) Baseline magnetic resonance imaging. (D, E) Intraoperative view of the lesions on the medial femoral condyle and trochlea. (F) Implantation of 4 aragonite-based implants. (G-I) Magnetic resonance imaging at 24-month follow-up. (J) Clinical scores trend.

Outcomes

The primary endpoint for this study was the change from baseline to 24 months in the mean overall KOOS. The overall KOOS was also measured during intermediate visits at 6, 12, and 18 months after treatment.

The study had 4 confirmatory secondary endpoints:

- Change in KOOS Pain score from baseline to month 24
- Change in KOOS Quality of Life score from baseline to month 24
- Change in KOOS Activities of Daily Living score from baseline to month 24
- Responder rate at month 24, defined as an improvement in overall KOOS $\geq$30

Patients were also evaluated with the IKDC subjective score.

All patients underwent MRI to assess the percentage of articular defect fill at 12 and 24 months after surgery. The following MRI protocol was adopted: field of view, 14 cm; slice thickness, 3 to 3.5 mm; matrix, 512 × 256 (or 384); and receiver bandwidth, 80 to 120 Hx/pixel. The sequences were as follows:

- Coronal intermediate-weighted fast spin echo (FSE), no fat saturation (FS), repetition time (TR) ≥3000 ms, echo time (TE) = 30-40 ms
- Coronal proton density–weighted FSE with FS, TR ≥3000 ms, TE = 10-20 ms
- Sagittal intermediate-weighted FSE, no FS, TR ≥3000 ms, TE = 30-40 ms
- Sagittal proton density–weighted FSE with FS, TR ≥3000 ms, TE = 10-20 ms
- Axial intermediate-weighted FSE, no FS, TR ≥3000 ms, TE = 30-40 ms
- Axial T2-weighted FSE with FS, TR ≥3000 ms, TE = ≥70 ms
- Sagittal T1-weighted FSE, no FS, TR = 600-800 ms, TE = 10-20 ms
- Oblique proton density–weighted FSE with FS, TR ≥3000 ms, TE = 10-20 ms, 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. On each MRI scan, 2 or 3 slices located within the implant on a sagittal scan and 2 or 3 slices located on a coronal scan were assessed. For each slice, the degree of cartilage defect volume fill was semiquantitatively assessed in increments of 25% (0%-24%, 25%-49%, 50%-74%, 75%-100%). In case of multiple implants/defects, a single range was calculated by averaging all implants in the same joint.

All clinical and radiologic data were collected by members of the medical staff of the involved site and archived in the study electronic case report form for analysis.

Statistical Analysis

Sample Size Calculation. The sample size was determined adaptively using a "Goldilocks" strategy[3] that incorporated an interim analysis to determine the appropriate sample size. The sample size selection procedure was designed to obtain approximately 80% power for a 2:1 randomization with an alternative hypothesis corresponding to an 8-point improvement in the overall KOOS at 24 months and assuming a 15% treatment failure rate in each study arm.

Outcome Analysis. The primary goal of the trial was to demonstrate superiority of the implant as compared with SSOC by testing the following hypothesis:

$$H_0: \theta_1 = \theta_0 \text{ vs } H_A: \theta_1 > \theta_0,$$

where θ_d was the mean response for arm d (d = 0 for SSOC and d = 1 for the implant). To test this hypothesis, the posterior probability of superiority was calculated: $\Pr(\theta_1 > \theta_0 \mid \text{data})$. The trial was considered a success if the posterior probability exceeded 0.98 at the final analysis. The 4 confirmatory secondary endpoints were prespecified to be tested in a hierarchical manner to control the type 1 error rate if primary superiority was demonstrated. Each secondary endpoint required a Bayesian posterior probability >0.975 for declaring superiority.

Treatment failures were defined as any secondary invasive intervention in the treated joint (eg, open, mini-open surgical, or arthroscopic procedures, as well as any intra-articular injection), regardless if related or unrelated to the original treatment. Baseline observation carried forward was applied to primary and secondary endpoints when a case was defined as a failure (ie, change from baseline assumed to be zero).

Covariate Analyses

Covariate analyses were completed for the primary and secondary confirmatory endpoints by including each covariate of interest in a mixed model for repeated measures (MMRM).[36] The prespecified covariates were age, presence of OA (evaluated by KL grade), and lesion size. It was further prespecified that an interaction $P < .15$ would be considered evidence supporting the presence of heterogeneity of treatment effects.

RESULTS

The first interim analysis was performed after 250 patients were enrolled, and it was reviewed by an external endpoint adjudication committee on November 22, 2019. The committee recommended stopping enrollment at that point and proceeding with 2-year follow-up owing to the anticipated success in the study. At the time of this analysis, 167 patients were included in the implant group, and 83 had been enrolled into the SSOC arm, consistent with the 2:1 randomization ratio. An additional patient was enrolled into the SSOC arm after the interim analysis, thus resulting in 84 patients (Figure 2). Patient characteristics and baseline scores were comparable between the treatment groups (Table 1). Three patients in the implant group and 1 in the SSOC group were later excluded from the full analysis set owing to detection of major entry violation exclusion criteria. Finally, 97% of patients completed the 2-year study.

Primary Endpoint

The primary endpoint was the change from baseline to 24 months in the mean overall KOOS. At baseline, there was a negligible difference in the implant and SSOC groups (mean difference, 0.5; 95% CI, 3.9-2.9). At the follow-up visits, the group differences in mean change values increased from 8.2 (95% CI, 3.3-13.0) at 6 months to 12.5 (95% CI, 7.3-17.8) at 12 months, 18.3 (95% CI, 13.0-23.5) at 18

TABLE 1
Characteristics of the 2 Treatment Groups[a]

	Agili-C		SSOC	
	No.	%	No.	%
Patients	167		84	
Male	107	64.1	51	60.7
Female	60	35.9	33	39.3
Age, y[b]	42 ± 11.2		46 ± 11.2	
Body mass index[b]	26.4 ± 4.2		27.9 ± 3.8	
≥30	37	22.2	27	32.1
<30	130	77.8	57	67.9
Tegner activity before onset of knee cartilage lesion				
Active, >4	132	79	61	72.6
Nonactive, ≤4	35	21	23	27.4
Age category, y				
≥50	40	24	34	40.5
<50	127	76	50	59.5
Age group, y				
21 to <45, young adulthood	94	56.3	41	48.8
45 to <65, middle adulthood	68	40.7	40	47.6
≥65, elderly	5	3	3	3.6
Smoking history				
Current	37	22.2	22	26.2
Past	22	13.2	17	20.2
Never	108	64.7	45	53.6
Kellgren-Lawrence grade of OA				
None: 0 or 1	91	54.5	30	35.7
Mild/moderate: 2 or 3	76	45.5	54	64.3
Lesion size >3 cm^2				
Yes	98	58.7	41	48.8
No	69	41.3	43	51.2
Single vs multiple lesions				
Single	109	65.3	58	69
Multiple	58	34.7	26	31
ICRS grade				
Osteochondral lesions: ICRS 4b	63	37.7	16	19
Chondral lesions: ICRS 3 and 4a	104	62.3	68	81
History of previous ACL reconstruction				
Yes	13	7.8	7	8.3
No	154	92.2	77	91.7
History of meniscectomy (medial/lateral)				
Yes	36	21.6	22	26.2
No	131	78.4	62	73.8
Concomitant meniscectomy (medial/lateral)				
Yes	50	29.9	19	22.6
No	117	70.1	65	77.4
Meniscal status				
Intact at the moment of surgery	94	56.3	44	52.4
History of partial meniscectomy	23	13.8	21	25
Concomitant surgery on meniscus	50	29.9	19	22.6

[a]ACL, anterior cruciate ligament; Agili-C, aragonite-based scaffold; ICRS, International Cartilage Regeneration & Joint Preservation Society; OA, osteoarthritis; SSOC, surgical standard of care.
[b]Mean ± SD.

months, and 22.5 (95% CI, 17.0-28.0) at 24 months. Overall, the implant group performed significantly better than SSOC at all time points, with an increasing advantage over time up to the final 24-month evaluation (Figure 3A). The Bayesian posterior probability of superiority was 1.000; therefore, it was concluded that the implant was superior to the SSOC.

Secondary Confirmatory Endpoints

The posterior probability of superiority for all 4 confirmatory secondary endpoints was 1.00. As this value was greater than the prespecified Bayesian posterior probability of 0.975, it was concluded that the implant was superior to the SSOC in the improvement from baseline to 24

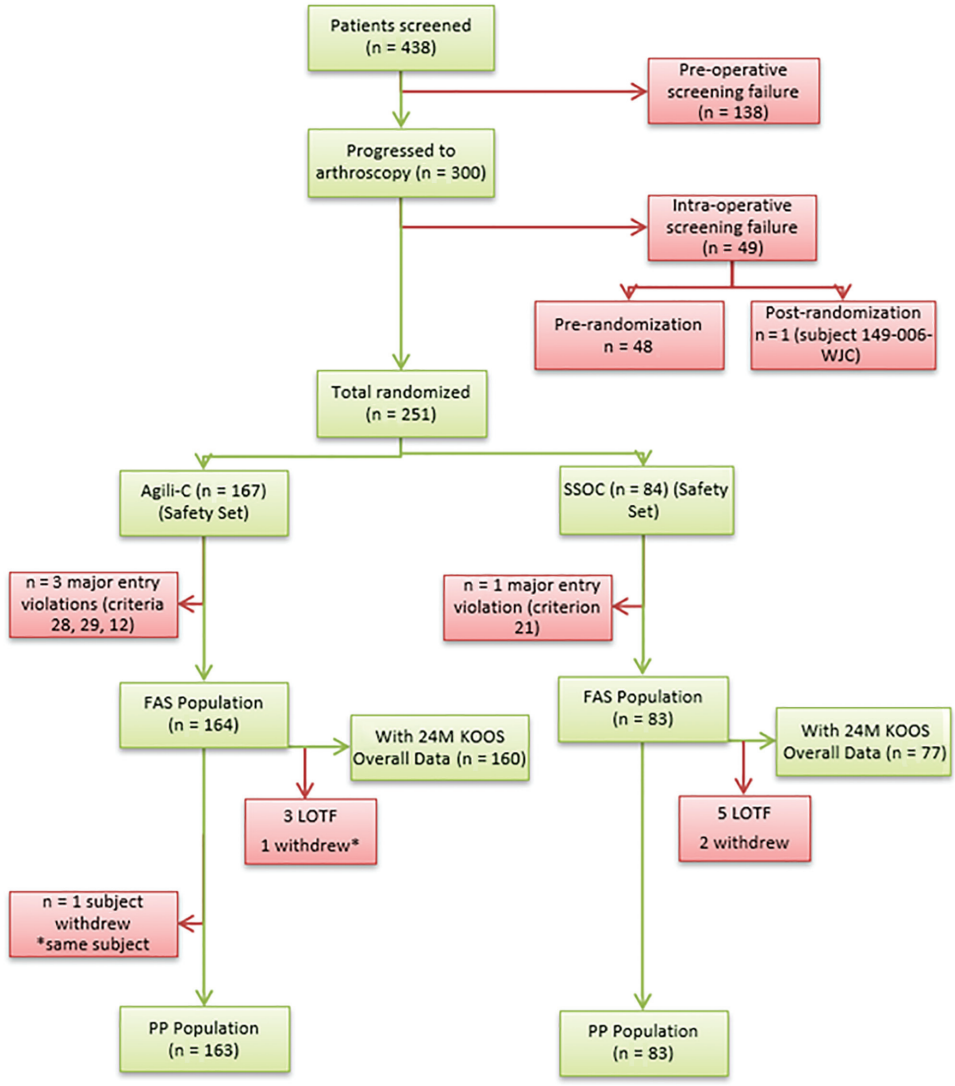


Figure 2. Patients' randomization flowchart and distribution between the treatment groups. 24M, 24 months; Agili-C, aragonite-based scaffold; FAS, full analysis set; KOOS, Knee injury and Osteoarthritis Outcome Score; LOTF, lost to follow-up; PP, per protocol; SSOC, surgical standard of care.

months in all of the secondary endpoints as well. These included the KOOS Pain, Quality of Life, and Activities of Daily Living subscales and the responder rate (ie, achieving a $\geq$30-point increase in the overall KOOS). Specifically, the responder rate was 77.8% for the implant group as compared with 33.6% for the SSOC group (Figure 3).

The minimal clinically important difference (MCID) for the IKDC has been reported as 16.7 at 12 months after articular cartilage repair surgery.[32] The IKDC change (mean $\pm$ SD) from baseline in the implant group was 24.0 $\pm$ 18.8 at 6 months, 32.5 $\pm$ 20.6 at 12 months, 38.1 $\pm$ 20.8 at 18 months, and 43.0 $\pm$ 21.2 at 24 months. The intergroup difference in mean values increased from 12.0 (95% CI, 6.5-17.5) at month 12 to 16.3 (95% CI, 10.7-21.9) at month 18 and up to 22.7 (95% CI, 16.8-28.6) at month 24. These results show that the IKDC scores are substantially higher than the MCID at each time point with significant superiority when compared with the control group ($P < .001$ for all time points), in line with the improvement in overall KOOS, assessed as the primary endpoint.

Missing Value Sensitivity Analysis

Follow-up compliance was very high: only 4 patients out of 164 (2.4%) in the implant group and 4 out of 83 (4.8%) in the SSOC group were missing the 24-month overall KOOS. A worst-case sensitivity analysis was conducted, in which missing values at every visit in the implant group were assigned the visit-specific minimum worst change in overall KOOS observed in this group and by assigning subject-specific maximum possible improvement to

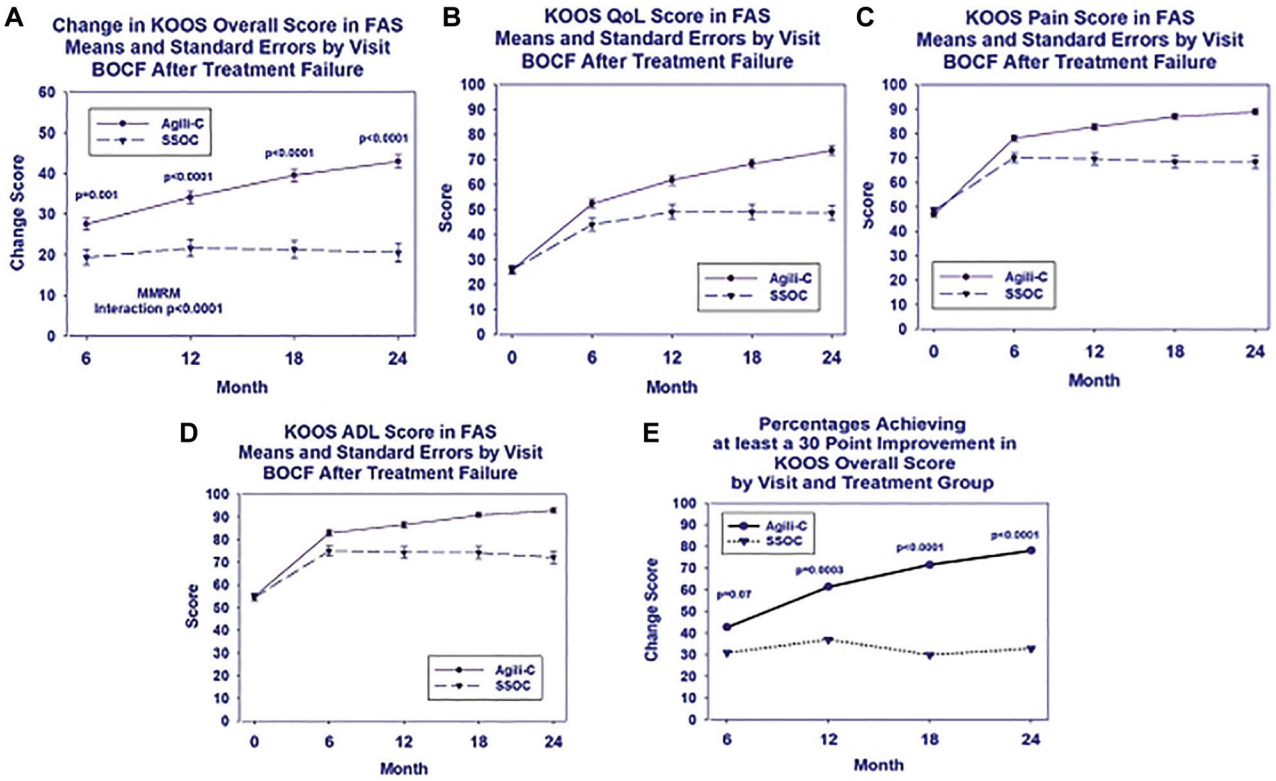


Figure 3. Primary and secondary endpoint data for Agili-C and SSOC over time. KOOS (A) overall and (B-D) QOL, Pain, and ADL subscales. (E) Percentage of responders (ie, ≥30-point improvement in overall KOOS). Values are presented as mean ± SE. ADL, Activities of Daily Living; Agili-C, aragonite-based scaffold; BOCF, baseline observation carried forward; FAS, full analysis set; KOOS, Knee injury and Osteoarthritis Outcome Score; MMRM, mixed model for repeated measures; QOL, Quality of Life; SSOC, surgical standard of care.

missing values in the SSOC group. The mean posterior distribution for the group difference in mean change from baseline to 24-month overall KOOS was 19.0 (95% CI, 13.8-25.1), and the posterior probability of superiority remained equal to 1.000. Therefore, the superiority conclusion based on the primary endpoint was very robust with regard to missing data.

Imaging Outcomes

MRI articular defect fill results demonstrated statistically significant differences between the treatment groups. At 24 months, 88.5% of those treated with the scaffold had ≥75% defect fill as opposed to only 30.9% among those treated with SSOC ($P < .0001$). Moreover, just 1.3% of the implant group had <50% defect fill at 24 months versus approximately 50% in the SSOC group (Table 2, Figure 1).

Failures

The rate of treatment failures was 21.4% (n = 18) in the SSOC group and 7.2% (n = 12) in the implant group. The difference was statistically significant according to an unadjusted chi-square test ($P = .002$). A higher failure rate was noted in the SSOC group with mild to moderate OA (27.8% of the patients) as compared with 5.3% in the

TABLE 2
Comparison of MRI Defect Fill at 12 and 24 Months Between Treatment Groups[a]

Time: MRI Defect Fill, %	Agili-C		SSOC		*P* Value[b]
	No.	%	No.	%	
Month 12					.0001
0-24	2	1.3	24	31.2	
25-49	2	1.3	13	16.9	
50-74	16	10.1	14	18.2	
75-99	107	67.7	17	22.1	
100	31	19.6	9	11.7	
Month 24					.0001
0-24	0	0.0	22	32.4	
25-49	2	1.3	12	17.6	
50-74	16	10.3	13	19.1	
75-99	95	60.9	14	20.6	
100	43	27.6	7	10.3	

[a]Agili-C, aragonite-based scaffold; MRI, magnetic resonance imaging; SSOC, surgical standard of care.
[b]Overall difference (Wilcoxon rank-sum test).

implant group. A similarly high failure rate was noted in the SSOC group with larger lesions (22.0%) when compared with the implant group (5.1%).

Safety and Adverse Events

Adverse event (AE) reporting was performed according to FDA standards and requirements. The overall AE rate was lower for the implant group, where 58.7% (98/167) of the patients experienced ≥1 AE, as compared with 77.4% (65/84) of the SSOC group. Although the AE rate might seem very high for the treatment and control groups, it must be noted that to meet FDA requirements, all the treatment-emergent AEs were listed, including unrelated events to the surgical procedure or the study device, such as COVID-19. The most common AE was increased transient knee pain following surgery, which occurred in 15.0% of the scaffold group versus 39.3% of the controls. Similar rates of increased swelling or effusion in the operated joint were reported between the treatment groups (5.4% in the scaffold group vs 4.8% in the control).

In terms of serious AEs, 2 patients (1.2%) in the scaffold group and 1 (1.2%) in the SSOC group experienced wound complications requiring antibiotics and prolonged wound dressing; 1 (0.6%) in the scaffold group developed septic arthritis requiring implant removal, surgical debridement, and antibiotic therapy; 2 (1.2%) in the scaffold group presented decreased range of motion of the index knee versus baseline; 2 (1.2%) in the scaffold group developed persistent muscle atrophy, which was still present at the final follow-up; 4 (4.8%) in the SSOC group presented OA progression leading to revision surgery; and 1 patient in each group developed deep venous thrombosis that was managed pharmacologically.

A complete data set concerning AEs is presented in the Appendix (Safety Evaluation; available in the online version of this article).

Covariate Analysis

Effect of the Presence of OA. In the implant group, 90 patients had no or minimal OA (KL 0 or 1), whereas 74 had mild or moderate OA (KL, 2 or 3). At 6 months, the overall increase in KOOS from baseline was 27.5 and 27.6, respectively, well beyond the 11.1 needed for an MCID. At the final 24-month follow-up, the mean increase was 43.9 in KL 0 or 1 and 41.9 in KL 2 or 3, without significant difference between these subgroups. In the SSOC group, 53 patients had no or minimal OA (KL 0 or 1), whereas 30 had mild or moderate OA (KL 2 or 3). The increase at 24 months was significantly lower in the moderate OA subgroup: 23.2 in KL 0 or 1 and 19.0 in KL 2 or 3. Based on the MMRM, the treatment group difference at 24 months was 18.7 (95% CI, 10.7-26.7; $P < .0001$) for KL 0 or 1 and 22.5 (95% CI, 15.6-29.3; $P < .0001$) for KL 2 or 3. There was no statistically reliable evidence that the superiority margin varied by degree of OA ($P = .476$).

Age. Patients were divided according to age (≥50 vs <50 years). In the implant group, 38 patients were ≥50 years and 126 were <50 years. For SSOC, 33 patients were ≥50 years and 50 were <50 years. Based on the MMRM, the treatment group difference at 24 months was 19.3 (95% CI, 10.2-28.5; $P < .0001$) for those ≥50 years and 21.8 (95% CI, 15.5-28.3; $P < .0001$) for those <50 years. There was no statistically reliable evidence that the superiority margin varied by age category ($P = .535$).

Lesion Size. Patients were divided according to the joint surface total lesion size (≤3 vs >3 cm^2). In the implant group, 68 had a total lesion size ≤3 cm^2, whereas 96 had >3 cm^2. For SSOC, 42 had a total lesion size ≤3 cm^2, whereas 41 had >3 cm^2. Based on the MMRM, the treatment group difference at 24 months was 25.0 (95% CI, 19.2-30.8; $P < .0001$) for those with a joint surface total lesion size ≤3 cm^2 and 17.9 (95% CI, 11.7-23.5; $P < .0001$) for those with a total lesion size >3 cm^2. Thus, there was a significantly larger treatment effect in larger defects.

DISCUSSION

The data collected in the present study support the superiority of the aragonite-based scaffold as compared with the current SSOC (ie, debridement/microfractures) in the treatment of knee joint surface lesions. These findings were confirmed by the high percentage of responders and low level of failures in the implant group. Based on covariate analysis, the improvement seen in the implant group remained robust regardless of age, lesion size, or presence of KL 2 or 3 OA. The strength of the study is the inclusion of patients with a broad spectrum of pathology, better representing the patient population encountered in real-world practice. In the available literature, other RCTs have compared cartilage regenerative approaches (ACI or osteochondral scaffolds) with microfracture. Recent systematic reviews and RCTs[17,18,26] failed to detect a significant difference in the clinical outcome between ACI and microfractures at mid- to long-term evaluation. Although ACI seems to provide better histologic features in the repair tissue, the clinical scores were similar when compared with microfracture, which, owing to lower costs and higher availability, still represents the SSOC for cartilage lesions.[34] Similarly, in terms of the comparison with osteochondral scaffolds, just 1 RCT of a hydroxyapatite-collagen scaffold is available,[21] and it showed no significant clinical difference, with the exception of a subgroup of patients with deep osteochondral defects. To date, the current main concern in the field of cartilage treatment is that there is no available treatment option that can effectively address a large population of patients belonging to different age groups with multiple lesions and concurrent joint problems, especially OA.[8] In addition, only a few trials described the outcomes of cartilage procedures performed in an osteoarthritic environment, where the high concentration of proinflammatory cytokines and catabolic agents may impair tissue repair.[10] Disappointing outcomes were recently published by Andriolo et al,[2] with a 59% cumulative failure rate at long term in 41 patients treated by ACI (KL 2 or 3). Two trials[7,33] investigated the use of

a hydroxyapatite-collagen scaffold in patients with early OA. Condello et al[7] documented a success rate of just 69% in a cohort of 26 patients evaluated for up to 3 years, whereas Sessa et al[33] evaluated 22 patients and reported a cumulative failure rate of 16.6%. Furthermore, MRI and computed tomography evaluations showed only slow and limited subchondral bone healing.[4]

Therefore, based on the literature, current surgical options are not able to sufficiently regenerate the complete osteochondral unit, except in the case of whole cold-stored osteochondral allograft transplants, which are not available in many parts of the world outside the United States. In contrast, the off-the-shelf aragonite-based scaffold employed in this trial was extensively tested in vitro and in the animal model,[5,23-25] showing its potential to effectively regenerate the cartilage layer and subchondral bone. Recent human studies also confirmed its safety and efficacy for treating chondral/osteochondral defects in multiple joints.[19,20]

The study design contains some limitations. Although the groups were very similar in most baseline characteristics—as the randomization was performed within strata defined by total lesion size, age category, and severity of OA, using variable block sizes—some parameters appeared unbalanced, even if not statistically significant between the groups. The prevalence of mild to moderate OA in the SSOC group was slightly higher than in the implant arm, but the implant arm included more patients affected by deep osteochondral defects (ICRS grade 4b) and more patients with larger lesions. Furthermore, the choice of debridement/microfracture as the control group, as suggested by FDA, leaves space for additional comparative studies against other approaches (ie, other osteochondral scaffolds or autologous chondrocyte transplantation) to further confirm the superiority of this scaffold. It is noteworthy to point out that patients with chondral/osteochondral defects often present "predisposing" factors that need to be addressed concurrently, such as limb malalignment, joint instability, and meniscal deficiency. It was initially requested to the FDA to allow the full range of common concomitant procedures in the study, but the FDA permitted only meniscal treatments, such as partial meniscectomy (n = 69; n = 50 in the scaffold group and n = 19 in the SSOC group, Table 1) and osteotomy (just 1 patient in the control group) and instructed against anterior cruciate ligament reconstruction to reduce the potential bias related to the contribution of concurrent anterior cruciate ligament procedures on the clinical outcome. This instruction allowed for a reliable comparison of the potential of the aragonite scaffold versus the SSOC. It is important to note that previous clinical studies on the aragonite scaffold included patients requiring complex treatments, such as osteotomies and ligament reconstruction, with positive clinical outcomes.[19]

CONCLUSION

The results of this study—the largest RCT currently available in the field of surgical cartilage repair—showed that the study device outperformed the SSOC in all the primary and secondary endpoints up to 24 months, even in patients in different age groups with mild to moderate OA and large articular cartilage lesions. Based on the present results, the implant was granted premarket approval by the FDA, providing the first FDA-approved scaffold for cartilage lesions in patients with focal defects as well as mild to moderate OA. Longer follow-up evaluations are required to ascertain the durability of these outcomes and to determine whether the aragonite-based scaffold might have long-term disease-modifying effects.

ACKNOWLEDGMENT

The authors thank all the people from the CLN Study Group.

REFERENCES

1. Andriolo L, Reale D, Di Martino A, Boffa A, Zaffagnini S, Filardo G. Cell-free scaffolds in cartilage knee surgery: a systematic review and meta-analysis of clinical evidence. *Cartilage*. 2021;12(3):277-292. doi:10.1177/1947603519852406
2. Andriolo L, Reale D, Di Martino A, et al. High rate of failure after matrix-assisted autologous chondrocyte transplantation in osteoarthritic knees at 15 years of follow-up. *Am J Sports Med*. 2019;47(9):2116-2122. doi:10.1177/0363546519855029
3. Broglio KR, Connor JT, Berry SM. Not too big, not too small: a goldilocks approach to sample size selection. *J Biopharm Stat*. 2014;24(3):685-705.
4. Christensen BB, Foldager CB, Jensen J, Jensen NC, Lind M. Poor osteochondral repair by a biomimetic collagen scaffold: 1- to 3-year clinical and radiological follow-up. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(7):2380-2387. doi:10.1007/s00167-015-3538-3
5. Chubinskaya S, Di Matteo B, Lovato L, Iacono F, Robinson D, Kon E. Agili-C implant promotes the regenerative capacity of articular cartilage defects in an ex vivo model. *Knee Surg Sports Traumatol Arthrosc*. 2019;27(6):1953-1964. doi:10.1007/s00167-018-5263-1
6. Collins NJ, Misra D, Felson DT, Crossley KM, Roos EM. Measures of knee function: International Knee Documentation Committee (IKDC) subjective Knee Evaluation Form, Knee Injury and Osteoarthritis Outcome Score (KOOS), Knee Injury and Osteoarthritis Outcome Score Physical Function Short Form (KOOS-PS), Knee Outcome Survey Activities of Daily Living Scale (KOS-ADL), Lysholm Knee Scoring Scale, Oxford Knee Score (OKS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Activity Rating Scale (ARS), and Tegner Activity Score (TAS). *Arthritis Care Res (Hoboken)*. 2011;63(suppl 11):S208-S228. doi:10.1002/acr.20632
7. Condello V, Filardo G, Madonna V, et al. Use of a biomimetic scaffold for the treatment of osteochondral lesions in early osteoarthritis. *Biomed Res Int*. 2018;2018:7937089. doi:10.1155/2018/7937089
8. de Windt TS, Vonk LA, Brittberg M, Saris DB. Treatment and prevention of (early) osteoarthritis using articular cartilage repair—fact or fiction? A systematic review. *Cartilage*. 2013;4(3):5S-12S.
9. Demers C, Hamdy C, Corsi K, Chellat F, Tabrizian M, Yahia L. Natural coral exoskeleton as a bone graft substitute: a review. *Biomed Mater Eng*. 2002;12(1):15-35.
10. Di Martino A, Kon E, Perdisa F, et al. Surgical treatment of early knee osteoarthritis with a cell-free osteochondral scaffold: results at 24 months of follow-up. *Injury*. 2015;46(suppl 8):S33-S38.
11. Ding C, Cicuttini F, Scott F, Cooley H, Boon C, Jones G. Natural history of knee cartilage defects and factors affecting change. *Arch Intern Med*. 2006;166(6):651-658. doi:10.1001/archinte.166.6.651

12. Food and Drug Administration. Osteoarthritis: structural endpoints for the development of drugs, devices, and biological products for treatment—draft guidance. Published 2018. Accessed August 1, 2022. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/osteoarthritis-structural-endpoints-development-drugs
13. Goyal D, Keyhani S, Lee EH, Hui JHP. Evidence-based status of microfracture technique: a systematic review of level I and II studies. *Arthroscopy*. 2013;29(9):1579-1588.
14. Heir S, Nerhus TK, Røtterud JH, et al. Focal cartilage defects in the knee impair quality of life as much as severe osteoarthritis: a comparison of knee injury and osteoarthritis outcome score in 4 patient categories scheduled for knee surgery. *Am J Sports Med*. 2010;38(2):231-237. doi:10.1177/0363546509352157
15. Hinckel BB, Thomas D, Vellios EE, et al. Algorithm for treatment of focal cartilage defects of the knee: classic and new procedures. *Cartilage*. 2021;13(1):473S-495S. doi:10.1177/1947603521993219
16. Hjelle K, Solheim E, Strand T, Muri R, Brittberg M. Articular cartilage defects in 1000 knee arthroscopies. *Arthroscopy*. 2002;18(7):730-734.
17. Kim J-H, Heo J-W, Lee D-H. Clinical and radiological outcomes after autologous matrix-induced chondrogenesis versus microfracture of the knee: a systematic review and meta-analysis with a minimum 2-year follow-up. *Orthop J Sports Med*. 2020;8(11):2325967120959280. doi:10.1177/2325967120959280
18. Knutsen G, Drogset JO, Engebretsen L, et al. A randomized multicenter trial comparing autologous chondrocyte implantation with microfracture: long-term follow-up at 14 to 15 years. *J Bone Joint Surg Am*. 2016;98(16):1332-1339. doi:10.2106/JBJS.15.01208
19. Kon E, Di Matteo B, Verdonk P, et al. Aragonite-based scaffold for the treatment of joint surface lesions in mild to moderate osteoarthritic knees: results of a 2-year multicenter prospective study. *Am J Sports Med*. 2021;49(3):588-598. doi:10.1177/0363546520981750
20. Kon E, Drobnic M, Davidson PA, Levy A, Zaslav KR, Robinson D. Chronic posttraumatic cartilage lesion of the knee treated with an acellular osteochondral-regenerating implant: case history with rehabilitation guidelines. *J Sport Rehabil*. 2014;23(3):270-275. doi:10.1123/jsr.2013-0054
21. Kon E, Filardo G, Brittberg M, et al. A multilayer biomaterial for osteochondral regeneration shows superiority vs microfractures for the treatment of osteochondral lesions in a multicentre randomized trial at 2 years. *Knee Surg Sports Traumatol Arthrosc*. 2018;26(9):2704-2715. doi:10.1007/s00167-017-4707-3
22. Kon E, Filardo G, Di Matteo B, Perdisa F, Marcacci M. Matrix assisted autologous chondrocyte transplantation for cartilage treatment: a systematic review. *Bone Joint Res*. 2013;1;2(2):18-25. doi:10.1302/2046-3758.22.2000092
23. Kon E, Filardo G, Robinson D, et al. Osteochondral regeneration using a novel aragonite-hyaluronate bi-phasic scaffold in a goat model. *Knee Surg Sports Traumatol Arthrosc*. 2014;22(6):1452-1464. doi:10.1007/s00167-013-2467-2
24. Kon E, Filardo G, Shani J, et al. Osteochondral regeneration with a novel aragonite-hyaluronate biphasic scaffold: up to 12-month follow-up study in a goat model. *J Orthop Surg Res*. 2015;10:81. doi:10.1186/s13018-015-0211-y
25. Kon E, Robinson D, Shani J, et al. Reconstruction of large osteochondral defects using a hemicondylar aragonite-based implant in a caprine model. *Arthroscopy*. 2020;36(7):1884-1894. doi:10.1016/j.arthro.2020.02.026
26. Kraeutler MJ, Belk JW, Purcell JM, McCarty EC. Microfracture versus autologous chondrocyte implantation for articular cartilage lesions in the knee: a systematic review of 5-year outcomes. *Am J Sports Med*. 2018;46(4):995-999. doi:10.1177/0363546517701912
27. Martin JA, Buckwalter JA. The role of chondrocyte senescence in the pathogenesis of osteoarthritis and in limiting cartilage repair. *J Bone Joint Surg Am*. 2003;85:106-110. doi:10.2106/00004623-200300002-00014
28. Matta C, Szücs-Somogyi C, Kon E, et al. Osteogenic differentiation of human bone marrow–derived mesenchymal stem cells is enhanced by an aragonite scaffold. *Differentiation*. 2019;107:24-34. doi:10.1016/j.diff.2019.05.002
29. Mithoeffer K, McAdams T, Williams R, Kreuz P, Mandelbaum B. Clinical efficacy of the microfracture technique for articular cartilage repair in the knee: an evidence-based systematic analysis. *Am J Sports Med*. 2009;37(10):2053-2063. doi:10.1177/0363546508328414
30. Niemeyer P, Feucht MJ, Fritz J, et al. Cartilage repair surgery for full-thickness defects of the knee in Germany: indications and epidemiological data from the German Cartilage Registry (KnorpelRegister DGOU). *Arch Orthop Trauma Surg*. 2016;136:891-897. doi.org/10.1007/s00402-016-2453-5
31. Osteoarthritis Research Society International. OARSI white paper—OA as a serious disease. 2018. https://www.oarsi.org/sites/default/files/library/2018/pdf/oarsi_white_paper_oa_serious_disease121416_1.pdf
32. Roos EM, Engelhart L, et al. Patient-reported outcome instruments for use in patients with articular cartilage defects. *Cartilage*. 2011;2(2):122-136.
33. Sessa A, Andriolo L, Di Martino A, et al. Cell-free osteochondral scaffold for the treatment of focal articular cartilage defects in early knee OA: 5 years' follow-up results. *J Clin Med*. 2019;14:8(11):e1978. doi:10.3390/jcm8111978
34. Solheim E, Krokeide AM, Melteig P, Larsen A, Strand T, Brittberg M. Symptoms and function in patients with articular cartilage lesions in 1000 knee arthroscopies. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(5):1610-1616. doi:10.1007/s00167-014-3472-9
35. Ulstei S, Arøen A, Røtterud JH, Løken S, Engebretsen L, Heir S. Microfracture technique versus osteochondral autologous transplantation mosaicplasty in patients with articular chondral lesions of the knee: a prospective randomized trial with long-term follow-up. *Knee Surg Sports Traumatol Arthrosc*. 2014;22(6):1207-1215. doi:10.1007/s00167-014-2843-6
36. Verbeke G, Molenberghs G. *Linear Mixed Models for Longitudinal Data*. Springer; 2000.
37. Weber AE, Locker PH, Mayer EN, et al. Clinical outcomes after microfracture of the knee: midterm follow-up. *Orthop J Sports Med*. 2018;9;6(2):2325967117753572. doi:10.1177/2325967117753572
38. Zaslav K, Cole B, Brewster R, et al; STAR Study Investigators. A prospective study of autologous chondrocyte implantation in patients with failed prior treatment for articular cartilage defects of the knee: the results of the STAR Clinical Trial. *Am J Sports Med*. 2009;37(1):42-55. doi:10.1177/0363546508322897
39. Zhang XX, He SH, Liang X, Li W, Li TF, Li DF. Aging, cell senescence, the pathogenesis and targeted therapies of osteoarthritis. *Front Pharmacol*. 2021;23;12:728100. doi:10.3389/fphar.2021.728100