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Microfractures and hydrogel scaffolds in the treatment of osteochondral knee defects: A clinical and histological evaluation

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ABSTRACT

Background: Osteochondral knee defects (OCD) are often symptomatic, causing pain and functional impairment even in young and active patients. Regenerative surgical options, aiming to stimulate natural cartilage healing, have been recently used as a first line treatment. In this study, a new hydrogel is investigated in its capacity to regenerate the ultra-structural quality of hyaline cartilage when combined with a classical microfracture technique.

Material and methods: Forty-six patients, affected by grade III and IV knee chondropathies, were consecutively treated between 2013 and 2015 with microfractures followed by application of a modern hydrogel in the lesion site. All patients underwent clinical evaluation (WOMAC) pre-operatively, at 6,12 and at 24 months postoperatively: the results were compared with a subsequent, consecutive, matched, control group of 23 patients treated with microfractures alone. In a parallel and separate in-vitro histological study, adipose derived mesenchymal stem cells (ADMSCs) were encapsulated in the hydrogel scaffold, induced to differentiation into chondrocytes, and observed for a 3 weeks period.

Results: The initial WOMAC score of 58.6 ± 11.0 in the study group was reduced by 88% at 6 months (7.1 ± 9.2) and 95% at 24 months (2.9 ± 5.9). The "in-vitro" study revealed a histological characterization typical of hyaline cartilage in study group. Separate biopsies performed at 12 months post-op in the study group also revealed type 2 collagen and hyaline-like cartilage in the regenerated tissue.

Conclusion: Our study demonstrated high patient satisfaction rates after microfractures combined with a modern hydrogel scaffold; histologic evaluation supported the hypothesis of creating an enhanced chondrogenic environment. Microfracture "augmentation" using modern acellular biomaterials, like hydrogels, might improve the clinical outcomes of this classical bone marrow stimulating procedure.

1. Introduction

Hyaline cartilage is critical to the natural function of the knee joint, resurfacing and lubricating its three compartments. The biomechanical properties of this delicate tissue are easily compromised by traumatic injuries and joint diseases due to its avascular structure and poor self-healing ability. When knee cartilage has been damaged, surgical restoration may be necessary: a recent clinical practice guideline by Cole et al[1] suggests three different variables driving decision making in surgical management of osteochondral lesions in the knee: size of the lesion (<3 cm^2 or >3 cm^2), location of the lesion (femoral condyles, tibial plateau or patellofemoral joint) and patient age. Despite decades of research, no alternative materials can truly replicate or substitute the biomechanical characteristics of the native knee cartilage. If regenerative options aim to stimulate natural healing by

Abbreviations: BMS, bone marrow stimulation; OCD, osteochondral defect; ACI, autologous chondrocyte implantation; MACI, mixed-assisted chondrocyte implantation; OAT, osteochondral autograft transfer; OCA, Osteochondral allograft transplantation; AMIC, Autologous Matrix Induced Chondrogenesis; BMS, bone marrow stem cells; PG/GC, polyglucosamine/glucosamine carbonate; BMI, body mass index; WOMAC, (Western Ontario and McMaster Universities Osteoarthritis Index); ASCs, adipose mesenchymal stem cells; hASCs, Human adipose-derived stromal/stem cells.

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fibrocartilage growth, other approaches are reconstructive employing osteochondral grafts or cartilage alone. Ultimately, the objective of many treatment methods is to produce a stable and congruent articular surface, restore function, and prevent the evolution of osteoarthritis in the knee.[2]

Microfracture surgery represents a traditional reparative strategy based on bone marrow stimulation (BMS). This arthroscopic technique attempts to achieve cartilage regeneration by exposing, through perforations, chondral lesions to mesenchymal stem cells migrating from the bone marrow. This approach is inexpensive and relatively non-invasive but unfortunately leads to formation of fibrocartilage rather than hyaline cartilage; this new tissue is usually more dense with less stiffness when compared with the normal hyaline cartilage.[3,4]

Alternatively, other surgical techniques that aim to reconstruct focal articular defects include osteochondral transfer (autograft), osteochondral transplantation (allograft) and isolated chondral implantation such as autologous chondrocyte implantation (ACI) or mixed-assisted chondrocyte implantation (MACI). The osteochondral autograft transfer (OAT) procedure transfers hyaline articular cartilage from a donor non-weight bearing site to fill an osteochondral defect (OCD) in the same knee. Matsusue et al[5] first described the mosaicplasty technique in 1993. Hangody et al[6] reported good to excellent clinical results in 92% of patients following femoral condylar transfers, 87% after tibial resurfacing and 74% after patellar and/or trochlear mosaicplasties. However, Solheim et al[7] reported variable clinical outcomes depending on age, sex, and size of the lesion. Increased failure rates were observed in female patients, patients older than 40 years and patients with defect sizes greater than 3 cm^2: the ideal candidate is a young patient with a unifocal less than 3 cm^2 full-thickness cartilage defect in the femoral condyle or trochlea.[8] Donor site morbidity is a significant limitation of the OAT procedure: this is particularly true in case of transferred grafts larger than 4 cm^2.[9] Success is also limited by the intrinsic biomechanical properties of the harvested cartilage that cannot withstand the forces of a higher load-bearing area, making it more susceptible to damage.[10] The technical challenges of fitting the osteochondral plug in the OCD to reproduce the normal depth and smoothness of the articular surface represents another major limitation of mosaicplasty.[11] Osteochondral allograft transplantation (OCA) is another option for treatment of OCD lesions larger than 2 or 3 cm^2. The goal of this procedure is to restore the normal cartilage surface using a size-matched cadaveric graft of hyaline cartilage supported by subchondral bone. Gross et al[12] demonstrated the value of fresh osteochondral allograft in reconstructing post-traumatic articular defects of the distal femur or proximal tibia in the young patient with survival rates up to 80% at 10 years for femoral condyle defects and 65% at 10 years for tibial plateau grafts. Jamali et al[13] showed less satisfactory results in the treatment of patellofemoral joint OCD lesions: the overall rate of good or excellent results was only 60%. Emmerson et al[14] confirmed that osteochondral allograft transplantation is a successful surgical technique in the treatment of osteochondritis dissecans of the femoral condyle, achieving good or excellent results in 70% of patients originally presenting with large defects (mean 7.5 cm^2) at long-term follow-up. On the other side, OCA procedures might stimulate an immunological response, usually within 3 weeks from transplantation: current studies report no need for immunosuppression during the healing process[15] but a slow trabecular incorporation of the transplanted allograft.[16,17] Furthermore, surgeons have to consider the risk of viral and bacterial transmission when using osteochondral allograft. In a review of 3500 anterior cruciate ligament reconstructions, the current authors demonstrated the same bacterial transmission risk between autografts and allografts.[18]

Recently, novel reparative modalities including autologous chondrocyte implantation (ACI) and mixed-assisted chondrocyte implantation (MACI) have been proposed for the treatment of early knee osteoarthritis. ACI is a two-stage procedure in which knee hyaline cartilage is first harvested by an arthroscopic biopsy and secondarily re-implanted after an in vitro expansion of the chondrocytes. Interestingly, Minas et al[19] suggested to avoid subsequent cartilage repair with autologous chondrocyte implantation in an area previously treated by the microfracture technique.

Mixed-assisted chondrocyte implantation (MACI) is a similar two-stage procedure using degradable chondrocyte-impregnated scaffolds. Despite advancements in tissue engineering, Mollon et al[20] concluded that insufficient evidence exists regarding the clinical and biomechanical superiority of modern tissue engineering methods over traditional techniques.

The objective of the current study is to present a surgical technique that combines a classical bone marrow stimulation technique (i.e., microfracture) with the use of a modern hydrogel scaffold matrix in a series of knees affected by osteochondral lesions (Modified Autologous Matrix Induced Chondrogenesis – AMIC). Hydrogels are promising, biocompatible materials used to replace chondral defects and demonstrated similar physical characteristics to the native hyaline cartilage, especially when implemented with bone marrow stem cells (BMS).[21] Different hydrogel scaffolds might be characterized by different reabsorption rates; when semi-permanent, chondrocyte overgrowth around the scaffold might cause an irregularity on the cartilaginous surface, but when the reabsorption rate is too fast, support for the migrating stem cells may be insufficient.

The current study reports preliminary clinical results using a modern polyglucosamine/glucosamine carbonate (PG/GC) based thermogelling injectable system: this hydrogel, when directly applied onto cartilage lesions, rapidly solidifies after being heated by the body temperature. The current authors utilized this new hydrogel to provide a scaffold matrix for chondrocyte proliferation following a standard microfracture procedure. To further investigate the potentiality and future applicability of the hydrogel used in the current study, in comparison to previous biomaterials, a separate tissue engineered histological study was conducted to evaluate the quality of the tissue generated by the application of adipose-derived mesenchymal stem cells (hASCs) encapsulated in this thermogelling system.

To the authors' knowledge, this is the first study reporting clinical and histological results of this modern thermogelling injectable system.

2. Materials and methods

Sixty-nine consecutive patients with OCD knee lesions, divided in two different treatments groups, were surgically treated at a single institution and enrolled in a matched pair study. All patients were treated by the same surgeon (GP) from April 2013 to June 2015. The first 46 patients were treated with microfracture surgery followed by the administration of the injectable thermogelling system (JointRep™ Oligo Medic Inc., Laval, Quebec Canada); twenty-three subsequent patients represented the control group and were treated with microfracture alone. The two groups were matched by age, gender, body mass index (BMI), and severity of the chondropathy as measured by the Outerbridge Classification.[23] The thermogelling system used in this study consisted of a combination of polyglucosamine and glucosamine carbonate (PG/GC). The Institutional Review Board (IRB) at the first author (GP) Institution approved this study.

Patients age ranged from 26 to 72 years. Patients affected by moderate to severe (Outerbridge III–IV) osteochondral lesions in the knee secondary to primary osteoarthritis or trauma and

refractory to conservative measures were included in the study. Patients with associated conditions such as previous partial meniscectomy, cruciate ligament lesions, or failed microfracture surgery (only in one case) were also included in the study and the associated procedures were performed simultaneously and in addition to the surgical treatment of the chondropathy Table 1.

Standard MRIs of the affected knee, identifying the chondropathic area, were obtained pre-operatively in all patients included in the study.

All arthroscopic procedures were performed following a regional anesthesia protocol. At the time of surgery, the knee was accessed through standard anteromedial and anterolateral arthroscopic portals. Once the lesion was identified and quantified [Table 1], the microfracture procedure was identically performed in both groups. Each microfracture averaged 8 mm in depth, 2 mm in diameter and was separated by at least 5 mm from the neighboring one. At this point, in the treatment group, arthroscopic irrigation was stopped and the thermogelling PG/GC system was delivered into the microfracture sites (Fig. 1). Differently from a previous study performed at the senior author Institution,[24] we did not use a chondroitin sulfate (CS) adhesive to physically immobilize this hydrogel because of its capability to rapidly solidify after being heated to body temperature.

Both groups followed the same postoperative rehabilitation protocol: all patients were first allowed to weight bear as tolerated (WBAT) immediately after the surgery: the use of a contralateral cane for 5–7 days postoperatively was suggested too. On postoperative day 15, the patients were allowed to start formal standard physical therapy, including quadriceps electro stimulation, swimming, and the use of a stationary bike for a reduced period of 3 weeks only.

All patients completed the WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index)[25] questionnaire prior to surgery and at 6, 12 and 24 months follow-up following the index procedure. An unpaired *t*-test was used to compare the average WOMAC scores and standard deviations (SD) between the experimental and control groups at time zero, at 6, 12 and 24 months from the index procedure.

A tissue engineered *in vitro* histological study, also approved by the local IRB, was separately conducted to evaluate the mechanism of integration of adipose derived mesenchymal stem cells (ASCs) in this hydrogel system. Human adipose-derived stromal/stem cells (hASCs) were isolated enzymatically from the lipoaspirate obtained from 6 healthy donors who underwent cosmetic liposuction surgery at the principal investigator's institution. Isolation was performed under sterile conditions. An equal volume of sterile-filtered 0.075% collagenase type II (Sigma-Aldrich Co., St. Louis, MO, USA) prepared in Dulbecco's Modified Eagle's Medium (DMEM, Lonza, Walkersville, MD, USA) was added to the lipoaspirate. The mixtures were incubated for 30 min at 37 °C with constant shaking. The samples were centrifuged at 400*g* for 10 min, after which the overlying fluid and adipose fractions were discarded.[26] The remaining stromal cell pellet was re-suspended and filtered through a 100 mm cell strainer. *In vitro* 3D cultures were established by encapsulation of hASCs in the PG/GC hydrogel at an initial concentration of 10^6 cells/ml. Two different groups were identified with 3 different samples in each group. The first group consisted of mesenchymal cells entrapped in the PG/GC hydrogel and maintained in basal culture medium. In the second group, the mesenchymal cells contained in the PG/GC hydrogel were induced towards the chondrogenic lineage using a commercially available chondrogenic medium (StemPro Chondrogenesis differentiation kit: Gibco, Invitrogen, Carlsbad, CA, USA). The differentiation protocol of the cultured cell-hydrogel systems was assessed after 3 weeks of *in vitro* culture using hematoxylin and eosin (H&E) and Alcian blue staining.

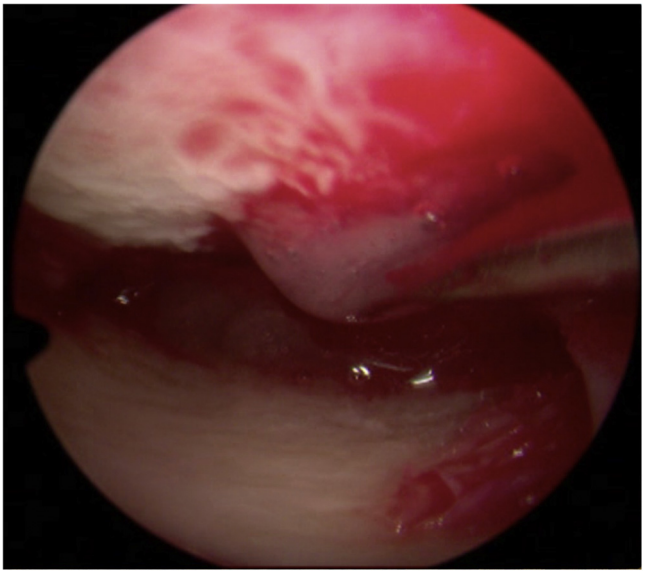

Fig. 1. Right Knee. Arthroscopic thermogelling application on the medial femoral condyle.

Table 1
Patients Clinical Evaluation: Statistics.

	Patient Statistics			
	Study Group		Control Group	
Total Number of Patients	N = 46		N = 23	
Patients included (WOMAC)	N = 46		N = 23	
Patients with WOMAC	N = 46		N = 23	
Patients, age	54.5 ± 9.5 (26–72)		56.6 ± 7.6 (44–70)	
Patients	Male	Female	Male	Female
	29 (63%)	17 (37%)	11 (47.8%)	12 (52.2%)
Treated Knee	Right	Left	Right	Left
	25 (54.3%)	21 (45.7%)	19 (69.6%)	4 (30.4%)
Grade	III	IV	III	IV
	10 (23%)	36 (78%)	6 (39%)	17 (61%)
Associated lesions	Lesion of Meniscus	Patellofemoral	Lesion of Meniscus	Patellofemoral
	98%	2%	100%	0%
Previous Microfracture	1	0	0	0
Average Defect size	2,8 cm	2,5 cm	2,7 cm	2,5 cm

3. Results

WOMAC scores were obtained preoperatively, at 6, 12 and 24 months follow-up. There were no statistically significant differences in preoperative WOMAC scores between the Microfracture + Hydrogels (experimental) and Microfracture alone (control) groups.

At 6 months follow-up, the overall WOMAC score and sub-scores significantly improved compared to preoperatively in both the control group and the study group. Furthermore, the study group also demonstrated a statistically significant improvement in the overall WOMAC score and in each sub-score when compared to the control group at 6 months.

At 24 months, patients in the control group undergoing microfracture alone demonstrated an overall improvement in postoperative WOMAC total scores and WOMAC physical sub-scores compared to preoperatively: anyway, there was no statistical difference in pre- versus postoperative pain in the control group while there was a statistically significant difference in all the other sub-scores. The study group demonstrated improvement in all scores at 24 months follow-up compared to preoperative: there was a statistically significant improvement in total WOMAC score and all sub-scores compared to the control group at 24 months. The mean WOMAC total score and sub-scores pre-operative, 6, 12 and 24 months post-operative are presented for both the study and control groups in Table 2. WOMAC total score and sub-scores of both clinical groups at 6, 12 and 24 months were statistically compared in Table 3.

Our in vitro histological study demonstrated apparent changes during the differentiation process: mesenchymal stem cells within the PG/GC hydrogel maintained in basal culture medium demonstrated classical fibroblastoid mesenchymal morphology, whereas induced mesenchymal cells in the PG/GC hydrogel cultured in chondrogenic medium displayed a more round morphology. Chondrogenically induced cells showed increased organization and neo-synthesis of the extracellular matrix. The induced samples exhibited an intense positivity for Alcian Blue staining, indicating the presence of increased glycosaminoglycans in the extracellular matrix. The differentiated cells were also noted to be trapped in characteristic small *lacunae*. Furthermore, this immunohistochemistry assay revealed the presence of human type II collagen (a specific marker for chondroblasts typically found in joint cartilage) in the extracellular matrix of the induced sample (Fig. 2).

During the follow-up period, two patients in the experimental group were dropped from the study following secondary trauma. One patient, a 59 year old male, required total knee arthroplasty 12 months after the previous arthroscopically surgery. At the time of the knee replacement surgery, the current authors were able to obtain the previously treated femoral condyle for histological evaluation. The femoral condyle was fixed in 10% neutral buffered formalin and after 48 h decalcified with EDTA 10% solution for 7 days. The specimen was then embedded in paraffin and approximately 3 μm osteochondral sections were cut. Histological slides were prepared with H&E and trichrome-blue stain in order to evaluate the integrity and restoration of the articular cartilage after treatment with microfracture and PG/GC hydrogel.

Prior to histological processing, the gross femoral bone-cartilage site was macroscopically examined. The specimen demonstrated the typical glass-like appearance of articular hyaline cartilage with restoration of the smooth white chondral surface of the distal femur. Histological examination revealed the well-organized complex structure typical of healthy articular cartilage without evidence of inflammation or fibrosis. The thin superficial zone was characterized by small, flattened chondrocytes with poor matrix and forms the gliding surface in contact with the synovial fluid; the deeper zones show larger and rounder chondrocytes that occur individually and in isogenous groups. Staining also revealed abundant extracellular matrix. The deepest zone contained calcified cartilage and subchondral bone demonstrated by intense red staining seen in Fig. 3(a–b). Immunohistochemistry assay for human type II collagen confirmed restoration of the hyaline cartilage. Strong positive expression of type II collagen was observed in the extracellular matrix of hyaline cartilage whereas

Table 2
Clinical Follow-up in Patients: WOMAC mean Scores.

	WOMAC Score and Sub-scores, mean (±SD)			
	Study Group		Control Group	
WOMAC, t = 0	N = 46		N = 23	
	Mean value (±SD)		Mean value (±SD)	
WOMAC Pain sub-score	12.6 (6.1)	–	7.9 (4.7)	–
WOMAC Stiffness sub-score	5.6 (3.1)	–	5.1 (2.1)	–
WOMAC Physical sub-score	38.1 (8.1)	–	41.7 (5.7)	–
WOMAC Total	58.6 (11.0)	–	56.5 (2.9)	–
WOMAC, t = 6 months	N = 46		N = 23	
	Mean value (±SD)	Mean reduction, % vs t = 0	Mean value (±SD)	Mean reduction, % vs t = 0
WOMAC Pain sub-score	1.3 (1.6)	90.0	2.7 (1.9)	65.4
WOMAC Stiffness sub-score	0.7 (1.0)	87.6	2.3 (0.9)	55.6
WOMAC Physical sub-score	4.9 (6.5)	87.1	22.3 (2.8)	46.6
WOMAC Total	7.1 (9.2)	88.0	28.4 (4.4)	49.8
WOMAC, t = 12 months	N = 46		N = 23	
	Mean value (±SD)	Mean reduction, % vs t = 0	Mean value (±SD)	Mean reduction, % vs t = 0
WOMAC Pain sub-score	1.0 (1.4)	92.4	6.4 (5.3)	19.2
WOMAC Stiffness sub-score	0.3 (0.6)	94.2	3.2 (1.9)	37.6
WOMAC Physical sub-score	3.0 (5.0)	92.1	31.1 (8.7)	25.5
WOMAC Total	4.2 (6.5)	92.9	41.9 (14.3)	26.0
WOMAC, t = 24 months	N = 44		N = 23	
	Mean value (±SD)	Mean reduction, % vs t = 0	Mean value (±SD)	Mean reduction, % vs t = 0
WOMAC Pain sub-score	0.5 (1.2)	96.4	7.8 (5.0)	1.6
WOMAC Stiffness sub-score	0.2 (0.5)	97.2	3.2 (1.1)	37.6
WOMAC Physical sub-score	2.1 (4.1)	94.4	35.4 (8.3)	15.2
WOMAC Total	2.9 (5.9)	95.1	48.3 (13.3)	14.5

Table 3
Statistical Analysis of WOMAC mean Scores.

	Control group t = 0 month	Control group t = 6 months	p value Control (0 vs 6)	Study group t = 0 month	Study group t = 6 months	p value Test (0 vs 6)	p value study vs Control (t = 0)	p value study vs Control (t = 6)
WOMAC Pain	7.9 (4.7)	2.7 (1.9)	<0.0001*	12.6 (6.1)	1.3 (1.6)	<0.0001*	0.0153*	0.0003*
WOMAC Stiffness	5.1 (2.1)	2.3 (0.9)	<0.0001*	5.6 (3.1)	0.7 (1.0)	<0.0001*	1.000**	<0.0001*
WOMAC Physical	41.7 (5.7)	22.3 (2.8)	<0.0001*	38.1 (8.1)	4.9 (6.5)	<0.0001*	0.3394**	<0.0001*
WOMAC Total	56.5 (2.9)	28.4 (4.4)	<0.0001*	58.6 (11.0)	7.1 (9.2)	<0.0001*	0.2846**	<0.0001*
	Control group t = 0 month	Control group t = 12 months	p value Control (0 vs 12)	Study group t = 0 month	Study group t = 12 months	p value Test (0 vs 12)	p value study vs Control (t = 0)	p value Study vs Control (t = 12)
WOMAC Pain	7.9 (4.7)	6.4 (5.3)	0.3154**	12.6 (6.1)	1.0 (1.4)	<0.0001*	0.0153*	<0.0001*
WOMAC Stiffness	5.1 (2.1)	3.2 (1.9)	0.0024**	5.6 (3.1)	0.3 (0.6)	<0.0001*	1.000**	<0.0001*
WOMAC Physical	41.7 (5.7)	31.1 (8.7)	<0.0001*	38.1 (8.1)	3.0 (5.0)	<0.0001*	0.3394**	<0.0001*
WOMAC Total	56.5 (2.9)	41.9 (14.3)	<0.0001*	58.6 (11.0)	4.2 (6.5)	<0.0001*	0.2846**	<0.0001*
	Control group t = 0 month	Control group t = 24 months	p value Control (0 vs 24)	Study group t = 0 month	Study group t = 24 months	p value Test (0 vs 24)	p value study vs Control (t = 0)	p value Study vs Control (t = 24)
WOMAC Pain	7.9 (4.7)	7.8 (5.0)	0.9446**	12.6 (6.1)	0.5 (1.2)	<0.0001*	0.0153*	<0.0001*
WOMAC Stiffness	5.1 (2.1)	3.2 (1.1)	0.0004*	5.6 (3.1)	0.2 (0.5)	<0.0001*	1.000**	<0.0001*
WOMAC Physical	41.7 (5.7)	35.4 (8.3)	0.044*	38.1 (8.1)	2.1 (4.1)	<0.0001*	0.3394**	<0.0001*
WOMAC Total	56.5 (2.9)	48.3 (13.3)	0.0060*	58.6 (11.0)	2.9 (5.9)	<0.0001*	0.2846**	<0.0001*

* Statistically significant difference (for two-tailed p value).
** No statistical difference.

sub-chondral bone demonstrated no expression of type II collagen Fig. 3(c–d).

4. Discussion

This study showed that patients treated with PG/GC thermogels in conjunction with standard microfractures demonstrated significant short-term improvements in pain, stiffness and function when compared to patients treated with microfractures alone. At the senior author Institution, a previous “in vivo” use of photo-reactive adhesive-hydrogel composites to repair human knee cartilage showed satisfactory short-term radiological and clinical results in a small group of patients.[24] This larger-scale clinical study confirmed that enhancing a classical microfracture technique with a PG/GC hydrogel biomaterial improves patient reported outcomes measurements (PROMs) up to 24 months from the index procedure.

Historically, bone marrow stimulation techniques such as microfracture surgery, originally proposed by Steadman in 1997, have proven to be an effective arthroscopic treatment for full-thickness chondral lesions in the knee.[27] This technique is cost-effective, technically simple, and carries an extremely low rate of associated patient morbidity. When applied in younger patients with small lesions, the microfracture technique demonstrated good to excellent long-term follow-up results in 67%–80% of patients.[28] This procedure is relatively contraindicated in patients over 50 years old and in patients with diffuse knee osteoarthritis. Factors affecting outcomes following microfracture include patient rehabilitation, knee alignment, and depth of the cartilage rim surrounding the lesion.[27]

Gobbi et al demonstrated worsening clinical outcomes at 2 and 5 years post microfracture treatment: severe postoperative degenerative changes were reported especially in older patients with multiple large lesions.[29] Other authors reported quite variable and high failure rates after microfracture surgery: 9% within 1 year, 18% within 3 years, and 32% within 5 years.[30] Nevertheless, the microfracture technique remains a good first line treatment option due to its simplicity and minimal invasiveness. Furthermore, its failure does not preclude subsequent procedures like OAT or ACI.[31,32] The major limitation of the microfracture technique remains related to the quality of the fibrocartilage tissue that is formed. The histological and biomechanical properties of fibrocartilage are inferior to the native hyaline cartilage.[3]

Because of this limitation, new biocompatible systems have been proposed to improve the quality of chondrogenic differentiation in conjunction with microfracture surgery. Hydrogels in particular have shown good potentials due to their lubricating quality and biomechanical features promoting the growth of stem cells. Hydrogels performance depend on their mechanical strength and elastic modulus. Early hydrogels had limited application due to strength and elastic properties insufficient to support physiologic loads at the knee.[10] Several preparative methods have been developed recently to improve these characteristics. Gao et al[33] developed nano-composite hydrogels formed by in-situ polymerization of acrylamide and exfoliated montmorillonite (MMT) layers as non-covalent cross-linkers: this composite demonstrated unprecedented elasticity, toughness, and self-healing. Mutos et al[34] used a biomimetic three-dimensional woven composite scaffold with properties that reproduced the anisotropy, viscoelasticity, and tension-compression nonlinearity of native articular cartilage. Bai et al[35] described a new preparative method utilizing freeze-casting and cryo-polymerization to create thermo-responsive composite hydrogels with an aligned macroporous structure that exhibit excellent mechanical properties. Shive et al[30] reported

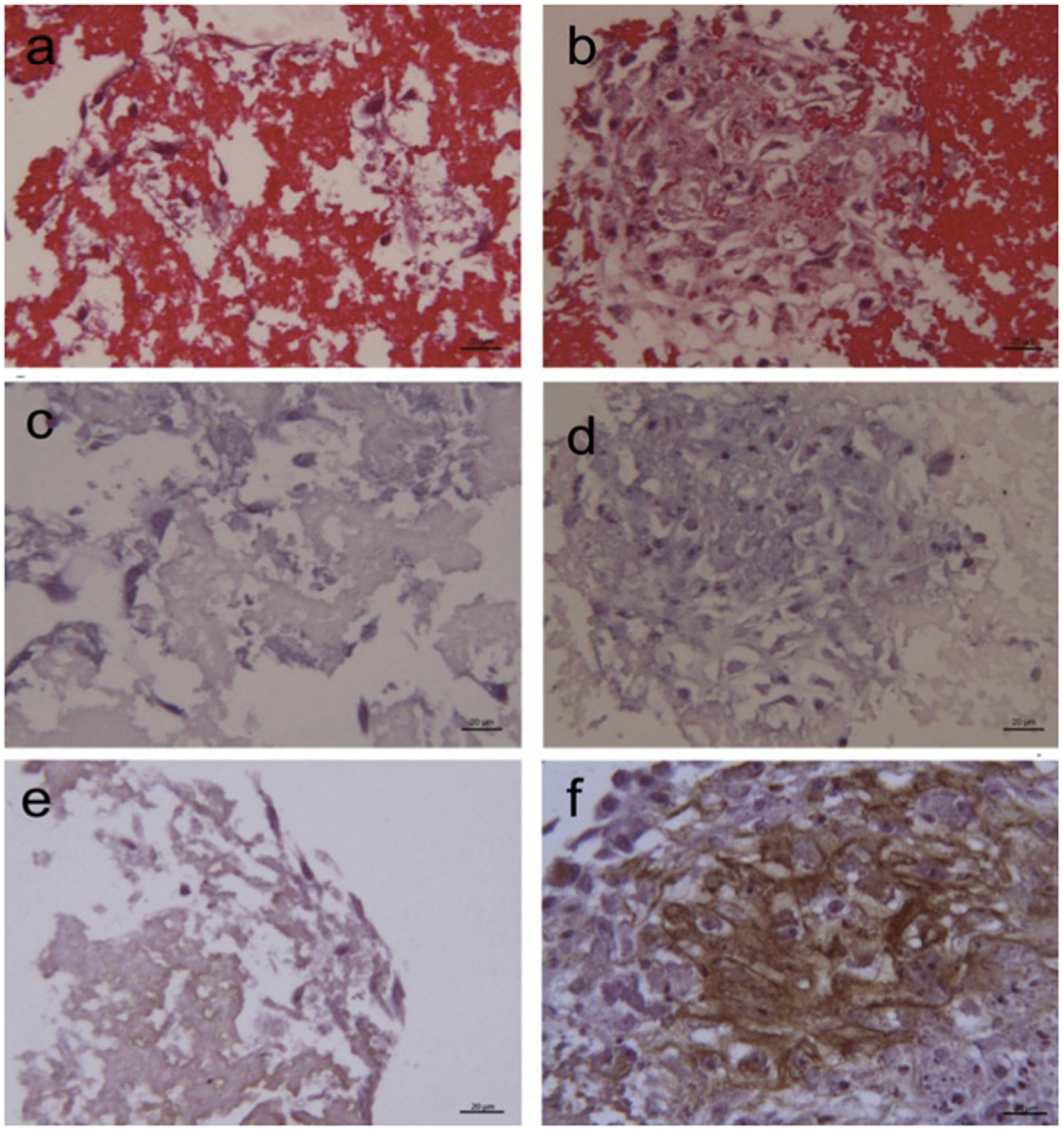

Fig. 2. Hematoxylin and eosin stain of control (a) and induced cells (b) seeded in *hydrogel PG/GC* solution, resembling a 3D scaffold culture system. Alcian blue staining of control (c) and induced (d) cells seeded in *hydrogel PG/GC* solution. Human collagen type II immunostaining negative in control samples (e) and positive in the extracellular matrix of induced cells (f).

early and mid-term clinical and radiological results using a chitosan scaffold (BST-CarGel, Piramal Life sciences, Bio-Orthopaedics Division) for cartilage repair in 34 knees affected by an isolated, focal (grade II to IV) cartilage lesion on the femoral condyle in comparison with 26 knees treated by only microfracture: blinded MRI analysis demonstrated a significantly greater treatment effect for lesion filling in the BST-CarGel group; on the other side, at five years F.U., there were no differences between the treatment groups according to the WOMAC subscales of pain, stiffness and function.

The current authors used a hydrogel composition that was determined by the final pH of the PG/GC solution and the thermogelling temperature. When poured in a test tube and incubated at 37 °C, the hydrogel solidified within one minute and showed an elastic and viscous modulus typical of a solid hydrogel: its lubricating mechanisms are multimodal, consisting of fluid pressurization-mediated lubrication and boundary lubrication as demonstrated by Muramaki et al[36] too. PG/GC hydrogels allow survival of nonadhesive cell types, such as chondrocytes, while discouraging adhesive cell growth (osteoblast, fibroblast), potentially promoting chondrogenesis.

This study supports autologous mixed–induced chondrogenesis, combining microfracture surgery with a hydrogel was a promising new technique for the treatment of chondral defects in the knee. This option combines the regenerative power of microfracture surgery with the capacity of biologic scaffolds to fill the defect. Our PROMs outcome showed statistically significant improvement in postoperative WOMAC total scores and WOMAC physical sub-scores compared to preoperatively and a statistically significant difference between the hydrogel + microfracture and the microfracture alone groups both at 12 months (92,4% vs 19.2% in pain reduction) as well as at 24 months (96,4% vs 1,6% in pain reduction) from the index procedure. The current results suggest three main considerations. First, our results differ from the Shive et al study[30]: those authors were not able to demonstrate any clinical difference between the hydrogel and microfracture alone groups at 5 years F.U. Differently from the current study, Shive et al[30] utilized a different glucosamine polysaccharide (Chitosan) to reinforce the post-microfracture blood clot. Secondarily, in the current study, after improvement for the first six months in all subgroups, a progressive and significant WOMAC score degradation was observed in the microfracture alone group. Third, the

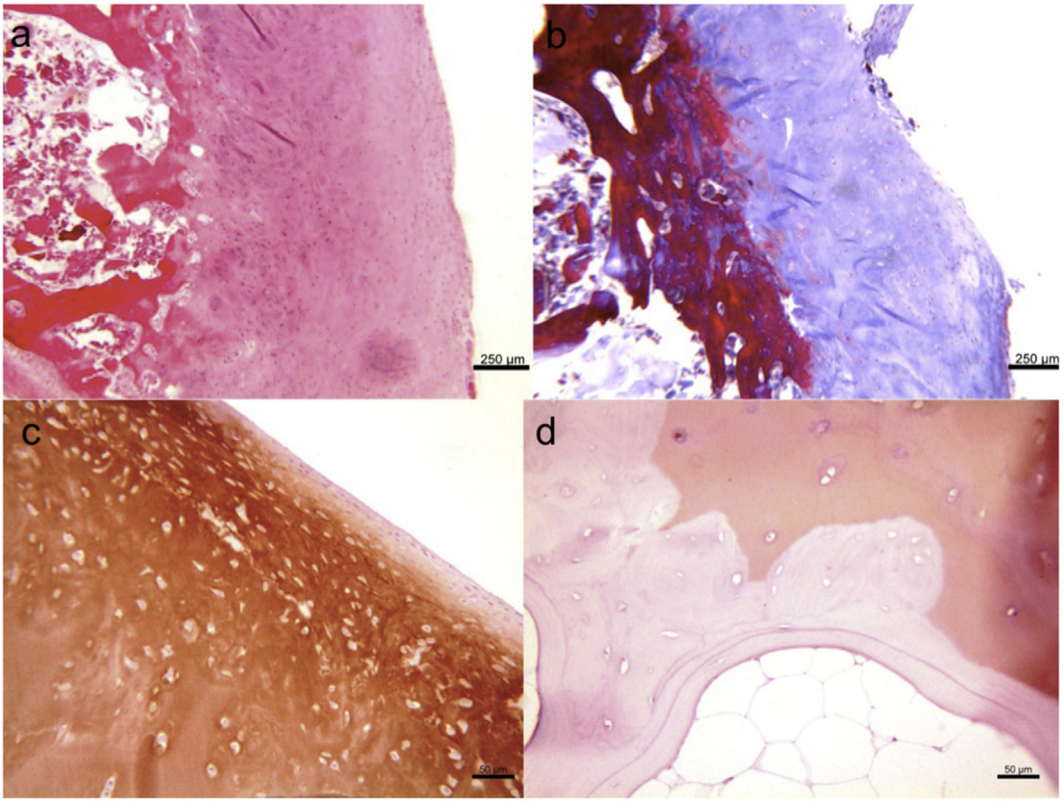

Fig. 3. (a) Hematoxylin and eosin stain of human femoral condyle section – 2.5X, (b) Trichrome Stain – 2.5X, (c) Human collagen type II immunostaining positive in the extracellular matrix of hyaline cartilage – 10X, (d) Human collagen type II immunostaining negative in the sub-chondral bone – 10X.

biomechanical properties of the tested thermogelling allowed for immediate postoperative weightbearing: the current authors rehabilitative protocol differs significantly from previously published rehabilitative protocols, when the full weightbearing status was not allowed for several weeks also in cases when modern hydrogels have been used.[37]

Several other studies have also shown significant clinical improvements in patients with osteochondral lesions treated with AMIC. A recent systematic review and meta-analysis conducted by Pot et al[38] demonstrated promising data in animal studies: those authors found that the implantation of acellular biomaterials after microfracture or subchondral drilling significantly improved cartilage regeneration by 15.6% compared to untreated empty defect controls. Furthermore, the addition of biologics to biomaterials significantly improved cartilage regeneration by 7.6% compared to control biomaterials. No significant differences were found between biomaterials of natural origin compared to synthetic scaffolds, hydrogels, and blends.

Gille et al[39] presented a case series of 32 chondral lesions in 27 patients treated with AMIC. In their study, the microfracture defects were covered with a collagen I/III matrix of porcine origin (Geistlich Pharma AG, Wolhusen, Switzerland) that was trimmed to fit the cartilage lesion. The mean defect size in this series was 4.2 cm^2 (all defects were classified as grade IV according to the Outerbridge classification) and clinical and radiological evaluations were performed at a mean follow-up of 32 months. Significant improvements were observed in all functional scores postoperatively with 87% of patients reporting high satisfaction. MRI analysis showed moderate to complete filling of the defects in most cases.

Kusano et al[40] reported significant clinical improvement in all functional scores in a case series of 38 patients treated with a collagen matrix biologic scaffold (Chondro-Gide, Geistlich, Wolhusen, Switzerland) for osteochondral lesions ranging in size from 2.3 to 4.4 cm^2: the mean follow-up in this study was 28.8 ± 1.5 months (range, 13–51 months). However, postoperative MRI evaluation showed the presence of tissue filling which was generally not complete or homogenous. Another study by Pascarella et al[41] analyzed 19 patients treated with a modified AMIC technique consisting of microfracture followed by coverage of the lesion with a biological collagen patch enriched with bone marrow blood drawn through the affected knee itself: a significant improvement in all scores and good MRI evidence of filling were observed at 2 years F.U.

A recent randomized control report from Volz et al[42] compared the radiological and clinical outcomes of the application of a biodegradable natural collagen type I/III membrane (Geistlich Pharma AG, Wolhusen, Switzerland) to treat mid-sized knee cartilage lesions (average, 3.6 cm^2) in three small groups of patients: at five years, 90–100% of the AMIC treated patients have improved to a normal or nearly normal functional status. The results of those studies[38,42] are very similar to those of the current study: both biomaterials (collagen I/III membranes and hydrogels) appear to guarantee appropriate mechanical properties to provide an environment supportive for cartilage formation.

The current study is not without limitations. The first limitation concerns the heterogenous, relatively small number of enrolled patients. Secondarily, though our outcomes are encouraging, longer follow-ups are needed to determine the long-term success of the proposed treatment. Third, the histologic study we performed independently from the patient population, using a chondrogenic induction method, does not necessarily support our clinical results.

5. Conclusion

Microfracture surgery is a traditional technique that has arguably become the gold standard in the treatment of osteochondral lesions of the knee especially in young patients. Microfracture "augmentation" using modern acellular biomaterials, like hydrogels, might improve the clinical outcomes of this bone marrow stimulating procedure. Our preliminary results demonstrate high patient satisfaction rates with minimal postoperative complications after microsurgery combined with a modern hydrogel scaffold and are consistent with previously reported series in the literature. The current modification of the original AMIC technique may improve cartilage repair outcomes and facilitate the intraoperative approach, but long-term studies are mandatory to confirm the reliability of this and others modified AMIC techniques.

Future investigations, comparing histological changes in knees treated with microfracture combined with hydrogels of different composition, would be useful to gain a better understanding of the effects of various scaffold chemical properties on articular cartilage. Whether or not pre-induced hydrogels can facilitate or accelerate chondrogenic differentiation of bone marrow stem cells and provide an advantage with respect to more traditional procedures remains an interesting debate.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval and consent to participate

All patients gave informed consent prior to being included into the study. All procedures involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments. The study was approved by the Research Ethics Committee of Casa di Cura Villa Regina, Bologna, Italy.

Consent for publication

All patients gave consent for publication.

Availability of supporting data

Please contact the contact author (PFI) for any required supporting data.

Authors' contributions

GP: surgeon; SR, EJW, DCV and PFI: Data gathering; FA and LB: Histologic evaluation.

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