J Mater Sci: Mater Med
DOI 10.1007/s10856-014-5188-2

A one-step treatment for chondral and osteochondral knee defects: clinical results of a biomimetic scaffold implantation at 2 years of follow-up

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Received: 21 December 2013 / Accepted: 25 February 2014

Abstract The increasing interest in the role of subchondral bone with regard to articular surface disease led to the development of new bioengineered strategies. Aim of this study is to evaluate the clinical and MRI outcome after the implantation of a nanostructured biomimetic three-phasic collagen–hydroxyapatite construct for the treatment of chondral and osteochondral defects of the knee in a large cohort of patients. Seventy-nine patients (63 M, 16 W), affected by grade III–IV femoral condyle or trochlea chondral lesions or osteochondritis dissecans (OCD) were consecutively treated. Mean age was 31.0 ± 11.3 years, mean lesion size was 3.2 ± 2.0 cm^2. Fifty patients underwent previous surgeries, concurrent procedures were necessary in 39 cases. The clinical outcome was evaluated using the IKDC and Tegner scores at 12 and 24 months of follow-up. At follow-up times an MRI was performed and evaluated with the MOCART score. All the scores improved significantly from the baseline. IKDC subjective score showed a further increase between 12 and 24 months of follow-up, and 82.2 % of the patients improved their symptoms at the final evaluation. Patients affected by OCDs had better results than those with degenerative lesions. Some abnormal MRI findings were present, even though no correlation was found with the clinical outcome. This one-step biomimetic approach developed to favor osteochondral tissue regeneration is effective in treating knees affected by damages of the articular surface, leading to a significant clinical improvement. However, abnormal MRI findings were present, even if not correlated with the clinical outcome.

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1 Introduction

The leading concept about the ideal biomaterial in orthopaedics is that an implant should mimic biology, architecture, and functional properties of the native tissue, favoring cell infiltration, attachment, proliferation, and differentiation into the new healing tissue. Biocompatibility and biodegradability through safe biochemical pathways at appropriate time intervals are also key, since the scaffold should support the early tissue formation and then undergo a gradual replacement by the regenerating tissue [1]. Finally, it should simplify the regenerative strategy overcoming the limits related to cell manipulation, thus being a ready-to-use product for clinical application: in fact, even though satisfactory clinical results have been documented for cell-based procedures at medium/long-term follow-up [2–4], the most recent trend is to choose techniques with a one-step implantation. Thus, one of the

Published online: 06 March 2014

emerging strategies for cartilage repair involves the implant of the sole biomaterial, able to induce "in situ" the resident bone marrow stem cells differentiation [1, 5] by providing the joint with the appropriate stimuli and produce orderly and durable tissue regeneration. The increasing interest in this one-step approach can be explained by both surgical and commercial standpoints, and a few studies showed promising results when treating cartilage defects [6–8]. However, the challenge is even more arduous when addressing defects of the articular surface were the subchondral bone is also affected, since bone and cartilage present intrinsic different nature and regenerative potential. Structural changes of the subchondral bone have been proved to be strictly involved in the pathogenic process of the chondral surface, and even focal cartilage defects can lead to changes of the underlying bone structure [9]. Moreover, some of the available reparative and regenerative techniques potentially lead to a relatively high incidence of subchondral bone alterations themselves [9–11] and present scarce indication for more complex lesions. Thus, a new bioengineered strategy for the treatment of the entire osteochondral unit has been developed, integrating distinct layers to address both cartilage and bone tissue regeneration and several contructs are being tested [12–14]. However, among the various osteochondral scaffolds documented in the preclinical setting, the results of just two of them have already been reported for their clinical use [1, 15, 16].

In this study we focused on the clinical and MRI evaluation of one of them. Following the concept of "biomimetism", the structure of this three-layered composite nanostructured biomaterial resembles the composition of the extracellular matrices of cartilage and bone tissue. After preclinical tests [17–19] it has been introduced into the clinical practice as a cell-free one-step treatment, and results of a pilot study on a small group of patients have been reported [20]. Aim of the present study is to evaluate the results after the implantation of this osteochondral biomimetic scaffold in a large group of patients affected by articular chondral and osteochondral lesions of the knee, documenting clinical and imaging findings, and analyzing the influence of possible prognostic factors.

2 Materials and methods

2.1 Patient selection

The present prospective clinical study was approved by the Hospital Ethics Committee and Internal Review Board, and informed consent was obtained from each patient.

Patients included were complaining of clinical symptoms like knee pain or swelling in association with grade III–IV (ICRS evaluation package) chondral and osteochondral lesions or osteochondritis dissecans (OCD) located at the femoral condyles or trochlea [21]. Exclusion criteria were: lesions at the patella or tibial plateaus, patients with non-corrected misalignment or instability of the knee. Patients presenting infectious, neoplastic, metabolic and inflammatory pathologies, as well as those not able to comply with the required post-operative rehabilitation regimen, were also excluded from this study. Conversely, patients with an axial deviation or an anterior cruciate ligament (ACL) lesion who underwent realignment or ligament reconstruction in the same surgical session as the scaffold implantation were included.

Eighty-two consecutive patients were consecutively enrolled and treated: three were lost to follow-up and 79 were prospectively evaluated at 12 and 24 months of follow-up. Among this group, 63 were men and 16 women, mean age was 31.0 ± 11.3 years. Three patients had multiple lesions, thus 82 defect sites were treated, located as follows: 41 medial femoral condyle (MFC), 26 lateral femoral condyle (LFC), and 15 at the trochlea level. Average size of the defects was 3.2 ± 2.0 cm^2. Etiology was micro traumatic or degenerative in 34 cases, traumatic in 11, and 34 patients had OCDs. Twenty-nine patients were surgically treated for the first time, whereas 50

Table 1 Detailed description of previous and combined surgery

Previous surgery ($n = 50$)	Combined surgery ($n = 39$)
28 Meniscectomies	11 Osteotomies
16 ACL reconstructions	- 6 High tibial osteotomies
14 Microfracturing	- 5 Distal femoral osteotomies
12 Shavings of chondral lesions	7 ACL reconstructions (hamstrings with "over the top" technique) [46]
11 Loose body removals	
3 Patellar fracture fixations	7 Meniscal scaffold implantations
2 Tibial plateau fixations	6 Meniscectomies
2 Synovial fold removals	4 Meniscal allograft implantations
2 Matrix-assisted ACIs	4 Microfracturing
1 Lateral release	3 Lateral releases
1 Re-fixation of an osteochondral fragment	2 Loose body removals
1 Patellar realignment	1 Patella realignment
1 Bursectomy	1 Osteochondral autograft implantation
1 Osteophyte removal	1 Meniscal repair
1 Mosaicplasty	1 Osteochondral fragment refixation
1 Osteochondral scaffold implantation	1 Patellar lateral facet removal.
1 LFC fixation	
1 Peroneal fracture treatment	

Fig. 1 Osteochondral biomimetic scaffold

patients had undergone previous surgeries (19 of them had previous cartilage surgery). In 39 patients other procedures were performed at the same time of scaffold implantation (Table 1).

2.2 Scaffold production [22]

The osteochondral biomimetic scaffold (Maioregen®, Fin-Ceramica Faenza S.p.A., Faenza, Italy) (Fig. 1) has a porous 3D composite structure, mimicking the whole osteochondral anatomy in three different layers. The deeper mineral phase is made of magnesium–hydroxyapatite (Mg–HA), directly nucleated onto collagen fibers during their self-assembling in proportion of 70 and 30 % of weight, respectively. Magnesium ions were added to increase the physicochemical, structural, and morphological affinities of the composite with newly formed natural bone [23]. The intermediate tidemark-like layer consists of a combination of Type I collagen (60 % of weight) and Mg–HA (40 % of weight), whereas the superficial cartilagine-like layer is entirely made of Type I collagen, with a smooth surface.

In detail, each layer is separately synthesized, starting from an atelocollagen aqueous solution (1 % w/w) in acetic acid, isolated from equine tendon. The upper non-mineralized chondral layer is obtained by dissolving 200 g of acetic solution of Type I collagen (Opocrin S.p.A., Corlo di Formigine, Modena, Italy) in 200 mL of bidistilled water, setting the pH at 5.5. The precipitate obtained is homogenized by adding 0.1 NaOH and rinsed in distilled water. The assembled collagen fibers are then cross-linked with 42 mL of 0.5 g/L 1,4-butanediol diglycidyl ether (BDDE) solution (Fluka, Sigma-Aldrich Group, St. Louis, MO) and stored at 4 °C for 48 h.

As occurs in the natural biological neo-ossification process, the intermediate and the lower layers were formed by nucleating bone-like nanostructured nonstoichiometric HA into self-assembling collagen fibers. The mineralized intermediate layer is obtained starting from two reagents: reagent A, prepared with Type I collagen acetic solution diluted with 300 g of H_3PO_4 40 mM, (final pH = 3.0); reagent B, prepared by mixing 480 mL of a 42 mM $Ca(OH)_2$ solution with 20 mL of 48 mM $MgCl_2 \bullet 6H_2O$ solution and 24 mL of SBF (Simulated Body Fluid). Under gentle stirring conditions, reagent A is dripped into reagent B until HA nanoparticles are nucleated into the autoassembled collagen fibers, reaching a final pH of 6.0. The resulting precipitate, composed of 60 % collagen and 40 % of HA, is rinsed in distilled water, cross-linked with 63 mL of BDDE crosslinking solution, and stored at 48 °C for 48 h. The lower layer is also prepared starting from two reagents: reagent C, obtained by adding to 200 g of Type I collagen acetic solution 40 mM H_3PO_4 achieving a pH of 3.0; reagent D, obtained by mixing 1,100 mL of 42 mM $Ca(OH)_2$ solution with 50 mL of 48 mM $MgCl_2 \bullet 6H_2O$ solution and 55 mL of SBF. Under stirring conditions, reagent C is dripped into reagent D until precipitation of HA occurs into auto-assembled collagen fibers, with a final pH of close to 7.0. The composite precipitate is 70 % HA and 30 % collagen, respectively. Afterwards, self-assembled collagen HA fibers are rinsed in bi-distilled water, cross-linked with 63 mL of BDDE solution and then stored at 48 °C for 48 h. The final construct is obtained by physically combining the layers on top of a Mylar sheet and then freeze dried and gamma-sterilized at 25 kGy.

The scaffold is finally chemically cross-linked through a biocompatible organic reticulation agent [24] to provide stability, thus increasing in situ hydrophilic properties and giving good handling properties, including flexibility.

2.3 Surgical procedure

The patient was positioned supine, under general or spinal anesthesia, with a pneumatic tourniquet around the proximal thigh. Through medial or lateral mini-arthrotomic parapatellar approach, the defects were exposed and prepared by removing the sclerotic subchondral bone with an osteotome. A lodge 8-mm deep with perpendicular shoulders was created to allow press-fit fixation of the implant. Stability was then visually tested by cyclic bending of the knee, both before and after tourniquet removal.

The post-operative care of the patient was managed with an early mobilization protocol, as previously described [20], in order to promote nutrition to the joint and defect healing, and to prevent the development of adhesions.

2.4 Post-operative rehabilitation protocol

Joint were early mobilized in order to help the resolution of swelling, to favor joint nutrition and healing, and to prevent the development of adhesions. At the same time patients performed early isometric and isotonic exercises, controlled mechanical compressions and at the discharge started voluntary muscular contraction and electrical neuromuscular stimulation (NMES). No weight-bearing and crutches were maintained for 3–4 weeks, then patients were allowed to progressively reach the full weight bearing. At 1–2 months swimming and cycling were allowed, starting low active functional training at 4–6 months, whereas no joint impact activities were allowed in the first 12 months.

2.5 Patients evaluation

Patients were prospectively evaluated preoperatively and postoperatively at 12 and 24 months of follow-up. The clinical outcome was assessed for each patient using the Cartilage Standard Evaluation Form as proposed by the ICRS (International Cartilage Repair Society) [25]. Moreover, a functional test of the knee was performed at each follow-up time, using the IKDC Knee Examination Form: the final functional grade of the knee (normal, nearly normal, abnormal or severely abnormal) was rated according to the lowest ratings in effusion and passive motion deficit [25]. The sport activity level was analyzed with the Tegner score and compared with pre-operative and pre-injury values [26].

Fifty patients (52 defects) also underwent MRI evaluation of the graft at 12 months and 45 (47 defects) at 24 months of follow-up. Examinations were carried out with a 1.5-T superconducting magnet (General Electric Co, Fairfield, Connecticut) with a dedicated quadrature detection knee coil (Quadknee; diameter, 18 cm), using the same sequences previously described for this imaging analysis [20]. The MOCART scoring system was applied for the evaluation of the implant at follow-up times [27]. The evaluation was performed in consensus by an orthopaedic surgeon and a musculoskeletal radiologist both experienced in cartilage procedures, who blindly assessed and reviewed the images.

2.6 Statistical analysis

All continuous data are expressed in terms of mean ± SD, categorical variables are expressed as proportions or percentages. The Kolmogorov–Smirnov test was performed to test normality of continuous variables. Repeated Measures GLM with post hoc Sidak correction for multiple comparisons was performed to compare normally distributed scores at the different follow-up times. The Friedman non-parametric test with Wilcoxon test post hoc test with Holm correction for multiple comparisons was performed to compare not normally distributed scores at the different follow-up times. The ANOVA test was performed to assess the between group differences of continuous and normally distributed and homoscedastic data; the Mann–Whitney test was used otherwise. The Spearman rank correlation was used to assess correlations between scores and continuous data. Fisher's exact test was performed to investigate the relationships between grouping variables. The analysis on the MRI findings were evaluated by the Monte Carlo method for small samples. For all tests $P < 0.05$ was considered significant.

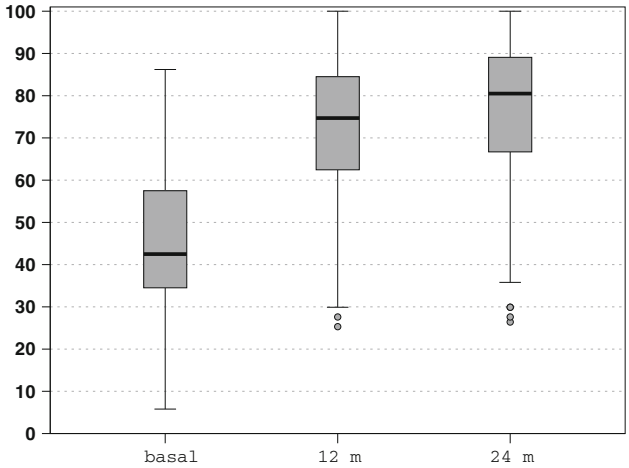


Fig. 2 IKDC subjective outcome (0–100) at basal level, 12 and 24 months of follow-up

3 Results

A statistically significant improvement in each of the clinical scores used was recorded between basal level and the 12 and 24 months' follow-ups.

The IKDC subjective score improved markedly from the baseline evaluation (47.4 ± 17.1) to the 12 month follow-up (72.1 ± 18.9; $P < 0.0005$) and further increased up to 24 months (76.2 ± 19.6) follow-up ($P = 0.004$) (Fig. 2). IKDC objective score changed from 72.1 % normal and nearly normal knees before the treatment (35 A, 22 B, 14 C and 8 D) to 88.6 % at 12 months' follow-up (44 A, 26 B, 4 C and 5 D; $P = 0.024$), and further improved at 24 months (57 A, 13 B, 6 C and 3 D; $P = 0.012$). The mean pre-injury Tegner score of 6.3 ± 2.2 decreased to 2.9 ± 2.0 pre-operatively, then improved to 3.8 ± 1.6 at the 12-months' follow-up and further raised to 4.4 ± 1.9 at 24 months. These results showed a statistically significant

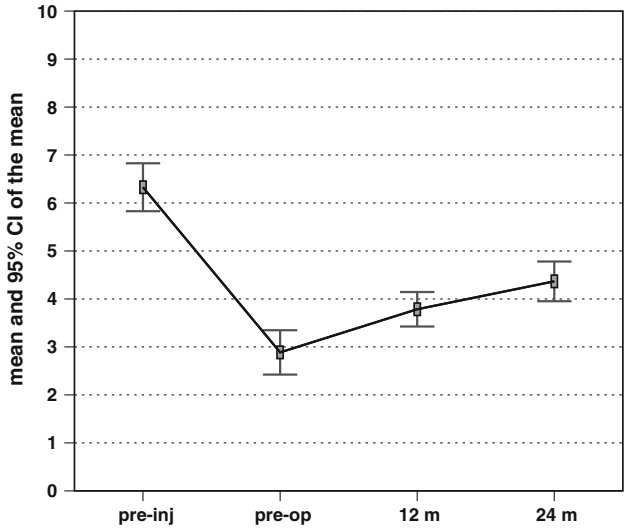


Fig. 3 Activity level evaluated with the Tegner score

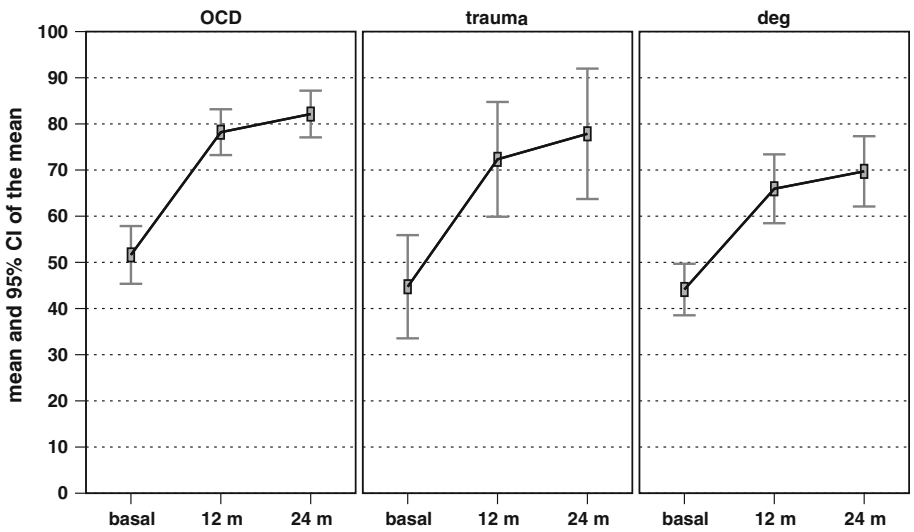
improvement ($P < 0.0005$) from the pre-operative level to the 12- and 24-months follow-ups; the further improvement between 12 and 24 months was also significant ($P = 0.004$); however, the final level of sport activity was lower than the pre-injury one (Fig. 3). Sixty-five patients (82.2 %) reported improvement of their symptoms at 24 months post-operative. Three patients (3.8 %) were considered failed, being re-operated for the same defect during the follow-up time.

Further analysis was performed to evaluate the parameters that might influence the clinical outcome: age, BMI, lesion area, lesion site, pre-injury activity level, sex, and the presence of previous or combined surgery did not significantly influence the clinical outcome in this series. Patients affected by OCDs had a higher IKDC subjective score than those with degenerative lesions at final follow-up ($P = 0.035$) (Fig. 4).

Seventeen patients (21.5 %) reported swelling at the treated knee in the early post-operative period, which in most of the cases resolved in a few days. Nine patients (11 %) needed an intervention due to joint stiffness. None of these events resulted to impair the final outcome.

Fifty-two lesions in 50 patients were evaluated with high resolution MRI at 12 months and 45 lesions (43 patients) at 24 months of follow-up. At 12 months the MOCART evaluation showed a complete filling of the cartilage area in 71.1 % of the lesions, complete integration of the graft in 71.1 % of cases, intact repair tissue surface in 40.4 % of the cases, homogeneous structure of the repair tissue in 42.3 % of cases, and iso-intense graft signal intensity score with the adjacent native cartilage in 51.9 and 50 % of the cases in dual T2-FSE and 3D-GE-FS sequences, respectively. Moreover, the subchondral bone appearance was considered normal in 38.5 %, whereas the lamina was not intact in all the cases. Finally, adhesions and effusion were shown in 0 and 51.9 % of the cases, respectively. At 24 months a complete filling of the cartilage was shown in 62.2 % of the lesions, complete integration of the graft was detected in 86.7 % of cases, the repair tissue surface was intact in71.1 %, the structure of the repair tissue was homogeneous in 48.9 % of the cases, and the graft signal intensity score was iso-intense with the adjacent native cartilage in 64.4 and 62.2 % of the cases in dual T2-FSE and 3D-GE-FS sequences, respectively. Moreover, the subchondral bone was intact in 33.3 % and the subchondral lamina in 4.4 % of the cases. Finally, adhesions and

Fig. 4 Etiology related results: OCD lesions present higher results, in particular in comparison with those obtained in the treatment of degenerative lesions



effusion were shown in 0 and 20 % of the cases, respectively. The total MOCART score showed an improvement between 12 and 24 months (25° $P = 55$, median = 70, 75° $P = 80$ vs 25° $P = 65$, median = 80, 75° $P = 90$; $P = 0.007$). No correlation was found in this series between MOCART variables and the clinical findings.

4 Discussion

The present study confirms in a large patient cohort that the use of this biomimetic three-layered implant is effective for the treatment of chondral and osteochondral knee lesions both in terms of clinical improvement and patient satisfaction. The IKDC subjective and Tegner scores documented a significant improvement at 12 months with respect to the pre-operative level, with a further increase up to 24 months of follow-up. Secondary finding is that MRI showed altered parameters that did not correlate with the clinical outcome at short-term of follow-up.

The current trend for the management of articular surface damage is to implant one-step a cell-free bio-construct into the defect site, able to exploit the self-regenerative potential of the host, reducing costs and morbidity of previous cell-based procedures. Moreover, since the subchondral bone has gained attention as primary factor in joint surface disease, specific scaffolds have been developed, combining different biomaterials in organized layers, aiming to reproduce the biological and functional requirements of bone and cartilage for the regeneration of both tissues. However, among the many osteochondral biomaterials tested in the preclinical setting [28–31], only two have been documented for the clinical application. A porous PLGA-calcium-sulfate bilayer biopolymer (TruFit, Smith & Nephew, Andover, MA), alternative to the mosaicplasty from a surgical point of view, produced controversial clinical results at short-term of follow-up, coupled with mostly mediocre MRI findings, and its use in the clinical practice is becoming questionable [16, 32–34].

The osteochondral scaffold used in this study has been produced following the concepts proposed by Tampieri et al. [22], who applied the new concept of biomimetism to design a composite graded hybrid biomaterial, which chemically and morphologically resembles the composition of the extra-cellular matrix of cartilage and bone tissues, respectively. This approach showed satisfactory and stable clinical results in case reports and pilot studies on small groups of selected patients [15, 20, 35–39]. Moreover, its effectiveness has been suggested also for OCD [37] and more compromised joints [36, 38, 40, 41] and even as a biological resurfacing procedure for selected osteoarthritic patients [39, 42]. On the other hand, rather unsatisfactory MRI results have been shown, which might be explained by the complexity of a treatment that aims at restoring the entire osteochondral unit, with two different tissues involved. Moreover, 8 mm deep slot into the subchondral bone are requested to host the scaffold. These factors probably lead to a slow regeneration process, and a slow improvement of the area treated has been observed over time up to 5 years [20]. The lack of correlation with the clinical outcome complies with most of the available literature: MRI has well documented limits in evaluating the results of cartilage procedures, as well as a predictive factor for the clinical outcome [43, 44]. Thus, while MRI still represents an essential diagnostic tool, the doubtful reliability with regards to the assessment of the quality of cartilage procedures makes patient function and symptoms still the primary outcome. The complexity of the regeneration processes led by the biomaterial mineral phase probably requires new MRI scoring systems focused also on the bone phase or CT scans for a better evaluation and understanding of tissue quality and clinical significance.

Even though satisfactory results have been documented in the heterogeneous patients of this study, some aspects of this procedure could be improved. The press-fit implantation technique may lead to a weak mechanical fixation in some cases [45], thus impairing the subsequent phases of integration and maturation of the scaffold. Thus, we recommend to carefully prepare the defect in order to produce an implantation site with perpendicular shoulders. However, it might still be not sufficient for a safe immediate joint mobilization, and improvements in fixation method are being considered. The insufficient stability of the scaffold might be a possible explanation to the above mentioned relatively high prevalence of post-operative swelling and joint-fibrosis, which could be caused by partial migration of some unstable micro-particles from the deeper phases of the implant into the joint space. However, specific studies should properly evaluate this aspect and possibly improve the implantation procedure. Finally, improvements in terms of physic-chemical composition might further improve the regeneration potential of this biomimetic approach.

The lack of a control group, and the presence of combined treatments are limits of the present study. The heterogeneity of the patients could be considered a limit as well, but on the other hand it allows to show that this biomimetic philosophy may represent a safe and effective regenerative option for the treatment of both chondral and osteochondral articular defects with large indications. Thus, this procedure can be considered a suitable option for several cases in which also the subchondral bone is involved in the damaged articular surface. Moreover, the implantation technique is easier and less expensive with respect to cell-based procedures.

Further randomized studies at longer follow-up are needed, comparing both clinical results and economic benefits to those of the other available procedures, in order to clarify potential and most suitable indications of this surgical approach. Ongoing studies are also focusing on the improvement of the scaffold properties through the functionalization of the biomaterial itself or its augmentation with cells or bioactive/anti-inflammatory molecules, and to limit the invasivity of the implantation technique while obtaining a better fixation to further improve the final clinical outcome.

5 Conclusion

The innovations in the field of biomaterials are providing the clinicians with new fascinating options to treat articular lesions. The implantation of this biomimetic scaffold to treat chondral and osteochondral knee defects proved to be effective in terms of clinical outcome at a short follow-up time in a large patient population, even though altered findings have been detected at MRI. Further randomized studies are needed to compare results and cost-effectiveness of this new treatment strategy with other available procedures, in order to clearly define potential and indications of this biomimetic nanostructured osteochondral scaffold for the regeneration of the articular surface.

Acknowledgments G. Altadonna, L. Andriolo, S. Bassini: II Clinic - Biomechanics Laboratory, Rizzoli Orthopaedic Institute, Bologna, Italy, E. Pignotti, K. Smith: Task Force, Rizzoli Orthopaedic Institute, Bologna, Italy.The study was performed at the Rizzoli Orthopaedic Institute, Bologna, Italy.

Conflict of interest All but two authors declare that they have no conflict of interest. Elizaveta Kon is paid consultant for CartiHeal (2009) Ltd, Israel and has stocks of CartiHeal (2009) Ltd, Israel, and she received fees for paid presentation from Finceramica SpA, Italy, and Fidia, Italy. Maurilio Marcacci has royalties and received research institutional support from Finceramica SpA, Italy.

References

1. Filardo G, Kon E, Roffi A, Di Martino A, Marcacci M. Scaffold-based repair for cartilage healing: a systematic review and technical note. Arthroscopy. 2013;29(1):174–86.
2. Filardo G, Kon E, Di Martino A, Iacono F, Marcacci M. Arthroscopic second-generation autologous chondrocyte implantation: a prospective 7-year follow-up study. Am J Sports Med. 2011; 39(10):2153–60.
3. Vasiliadis HS, Danielson B, Ljungberg M, McKeon B, Lindahl A, Peterson L. Autologous chondrocyte implantation in cartilage lesions of the knee: long-term evaluation with magnetic resonance imaging and delayed gadolinium-enhanced magnetic resonance imaging technique. Am J Sports Med. 2010;38(5):943–9.
4. Peterson L, Vasiliadis HS, Brittberg M, Lindahl A. Autologous chondrocyte implantation: a long-term follow-up. Am J Sports Med. 2010;38(6):1117–24.
5. Kon E, Filardo G, Roffi A, Andriolo L, Marcacci M. New trends for knee cartilage regeneration: from cell-free scaffolds to mesenchymal stem cells. Curr Rev Musculoskelet Med. 2012;5(3): 236–43.
6. Dhollander AA, De Neve F, Almqvist KF, Verdonk R, Lambrecht S, Elewaut D, Verbruggen G, et al. Autologous matrix-induced chondrogenesis combined with platelet-rich plasma gel: technical description and a five pilot patients report. Knee Surg Sports Traumatol Arthrosc. 2011;19(4):536–42.
7. Pascarella A, Ciatti R, Pascarella F, Latte C, Di Salvatore MG, Liguori L, Iannella G. Treatment of articular cartilage lesions of the knee joint using a modified AMIC technique. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):509–13.
8. Schiavone Panni A, Cerciello S, Vasso M. The manangement of knee cartilage defects with modified amic technique: preliminary results. Int J Immunopathol Pharmacol. 2011;24(1 Suppl 2): 149–52.
9. Pape D, Filardo G, Kon E, van Dijk CN, Madry H. Disease-specific clinical problems associated with the subchondral bone. Knee Surg Sports Traumatol Arthrosc. 2010;18(4):448–62.
10. Filardo G, Kon E, Di Martino A, Perdisa F, Busacca M, Tentoni F, Balboni F et al. Is the clinical outcome after cartilage treatment affected by subchondral bone edema? Knee Surg Sports Traumatol Arthrosc. 2013.
11. Minas T, Von Keudell A, Bryant T, Gomoll AH. The John Insall Award: a minimum 10-year outcome study of autologous chondrocyte implantation. Clin Orthop Relat Res. 2014;472(1):41–51.
12. Martin I, Miot S, Barbero A, Jakob M, Wendt D. Osteochondral tissue engineering. J Biomech. 2007;40(4):750–65.
13. Schek RM, Taboas JM, Segvich SJ, Hollister SJ, Krebsbach PH. Engineered osteochondral grafts using biphasic composite solid free-form fabricated scaffolds. Tissue Eng. 2004;10(9–10): 1376–85.
14. Sherwood JK, Riley SL, Palazzolo R, Brown SC, Monkhouse DC, Coates M, Griffith LG, et al. A three-dimensional osteochondral composite scaffold for articular cartilage repair. Biomaterials. 2002;23(24):4739–51.
15. Kon E, Delcogliano M, Filardo G, Busacca M, Di Martino A, Marcacci M. Novel nano-composite multilayered biomaterial for osteochondral regeneration: a pilot clinical trial. Am J Sports Med. 2011;39(6):1180–90.
16. Melton JT, Wilson AJ, Chapman-Sheath P, Cossey AJ. TruFit CB bone plug: chondral repair, scaffold design, surgical technique and early experiences. Expert Rev Med Devices. 2010;7(3):333–41.
17. Kon E, Mutini A, Arcangeli E, Delcogliano M, Filardo G, Nicoli Aldini N, Pressato D, et al. Novel nanostructured scaffold for osteochondral regeneration: pilot study in horses. J Tissue Eng Regen Med. 2010;4(4):300–8.
18. Kon E, Delcogliano M, Filardo G, Fini M, Giavaresi G, Francioli S, Martin I, et al. Orderly osteochondral regeneration in a sheep model using a novel nano-composite multilayered biomaterial. J Orthop Res. 2010;28(1):116–24.
19. Kon E, Filardo G, Delcogliano M, Fini M, Salamanna F, Giavaresi G, Martin I, et al. Platelet autologous growth factors decrease the osteochondral regeneration capability of a collagen-hydroxyapatite scaffold in a sheep model. BMC Musculoskelet Disord. 2010;11:220.
20. Kon E, Filardo G, Di Martino A, Busacca M, Moio A, Perdisa F, Marcacci M. Clinical results and MRI evolution of a nano-composite multilayered biomaterial for osteochondral regeneration at 5 years. Am J Sports Med. 2013.
21. ICRS. Cartilage injury evaluation package. 2000. http://www.cartilage.org/Evaluation_Package/ICRS_Evaluation.pdf.
22. Tampieri A, Sandri M, Landi E, Pressato D, Francioli S, Quarto R, Martin I. Design of graded biomimetic osteochondral composite scaffolds. Biomaterials. 2008;29(26):3539–46.

23. Serre CM, Papillard M, Chavassieux P, Voegel JC, Boivin G. Influence of magnesium substitution on a collagen-apatite biomaterial on the production of a calcifying matrix by human osteoblasts. J Biomed Mater Res. 1998;42(4):626–33.
24. Zeeman R, Dijkstra PJ, van Wachem PB, van Luyn MJ, Hendriks M, Cahalan PT, Feijen J. The kinetics of 1,4-butanediol diglycidyl ether crosslinking of dermal sheep collagen. J Biomed Mater Res. 2000;51(4):541–8.
25. ICRS. ICRS Cartilage injury evaluation package. 2000.
26. Tegner Y, Lysholm J. Rating systems in the evaluation of knee ligament injuries. Clin Orthop Relat Res. 1985;198:43–9.
27. Marlovits S, Striessnig G, Resinger CT, Aldrian SM, Vecsei V, Imhof H, Trattnig S. Definition of pertinent parameters for the evaluation of articular cartilage repair tissue with high-resolution magnetic resonance imaging. Eur J Radiol. 2004;52(3):310–9.
28. Liu M, Yu X, Huang F, Cen S, Zhong G, Xiang Z. Tissue engineering stratified scaffolds for articular cartilage and subchondral bone defects repair. Orthopedics. 2013;36(11):868–73.
29. Marmotti A, Bruzzone M, Bonasia DE, Castoldi F, Von Degerfeld MM, Bignardi C, Mattia S, et al. Autologous cartilage fragments in a composite scaffold for one stage osteochondral repair in a goat model. Eur Cell Mater. 2013;26:15–32.
30. Gotterbarm T, Breusch SJ, Jung M, Streich N, Wiltfang J, Berardi Vilei S, Richter W et al. Complete subchondral bone defect regeneration with a tricalcium phosphate collagen implant and osteoinductive growth factors: a randomized controlled study in Gottingen minipigs. J Biomed Mater Res B Appl Biomater. 2013.
31. Kon E, Filardo G, Robinson D, Eisman JA, Levy A, Zaslav K, Shani J et al. Osteochondral regeneration using a novel aragonite-hyaluronate bi-phasic scaffold in a goat model. Knee Surg Sports Traumatol Arthrosc. 2013.
32. Carmont MR, Carey-Smith R, Saithna A, Dhillon M, Thompson P, Spalding T. Delayed incorporation of a TruFit plug: perseverance is recommended. Arthroscopy. 2009;25(7):810–4.
33. Joshi N, Reverte-Vinaixa M, Diaz-Ferreiro EW, Dominguez-Oronoz R. Synthetic resorbable scaffolds for the treatment of isolated patellofemoral cartilage defects in young patients: magnetic resonance imaging and clinical evaluation. Am J Sports Med. 2012;40(6):1289–95.
34. Dhollander AA, Liekens K, Almqvist KF, Verdonk R, Lambrecht S, Elewaut D, Verbruggen G, et al. A pilot study of the use of an osteochondral scaffold plug for cartilage repair in the knee and how to deal with early clinical failures. Arthroscopy. 2012;28(2):225–33.
35. Delcogliano M, de Caro F, Scaravella E, Ziveri G, De Biase CF, Marotta D, Marenghi P et al. Use of innovative biomimetic scaffold in the treatment for large osteochondral lesions of the knee. Knee Surg Sports Traumatol Arthrosc. 2013.
36. Filardo G, Di Martino A, Kon E, Delcogliano M, Marcacci M. Midterm results of a combined biological and mechanical approach for the treatment of a complex knee lesion. Cartilage (3). 2012.
37. Filardo G, Kon E, Di Martino A, Busacca M, Altadonna G, Marcacci M. Treatment of knee osteochondritis dissecans with a cell-free biomimetic osteochondral scaffold: clinical and imaging evaluation at 2-year follow-up. Am J Sports Med. 2013;41(8):1786–93.
38. Kon E, Delcogliano M, Filardo G, Altadonna G, Marcacci M. Novel nano-composite multi-layered biomaterial for the treatment of multifocal degenerative cartilage lesions. Knee Surg Sports Traumatol Arthrosc. 2009;17(11):1312–5.
39. Marcacci M, Zaffagnini S, Kon E, Marcheggiani Muccioli GM, Di Martino A, Di Matteo B, Bonanzinga T, et al. Unicompartmental osteoarthritis: an integrated biomechanical and biological approach as alternative to metal resurfacing. Knee Surg Sports Traumatol Arthrosc. 2013;21(11):2509–17.
40. Filardo G, Kon E, Perdisa F, Di Matteo B, Di Martino A, Iacono F, Zaffagnini S, et al. Osteochondral scaffold reconstruction for complex knee lesions: a comparative evaluation. Knee. 2013;20(6):570–6.
41. Perdisa F, Filardo G, Di Matteo B, Di Martino A, Marcacci M. Biological knee reconstruction: a case report of an Olympic athlete. Eur Rev Med Pharmacol Sci. 2013; 17(Suppl 3).
42. Gomoll AH, Filardo G, de Girolamo L, Espregueira-Mendes J, Marcacci M, Rodkey WG, Steadman JR, et al. Surgical treatment for early osteoarthritis. Part I: cartilage repair procedures. Knee Surg Sports Traumatol Arthrosc. 2012;20(3):450–66.
43. de Windt TS, Welsch GH, Brittberg M, Vonk LA, Marlovits S, Trattnig S, Saris DB. Is magnetic resonance imaging reliable in predicting clinical outcome after articular cartilage repair of the knee?: a systematic review and meta-analysis. Am J Sports Med. 2013;41(7):1695–702.
44. Blackman AJ, Smith MV, Flanigan DC, Matava MJ, Wright RW, Brophy RH. Correlation between magnetic resonance imaging and clinical outcomes after cartilage repair surgery in the knee: a systematic review and meta-analysis. Am J Sports Med. 2013;41(6):1426–34.
45. Kon E, Delcogliano M, Filardo G, Pressato D, Busacca M, Grigolo B, Desando G, et al. A novel nano-composite multilayered biomaterial for treatment of osteochondral lesions: technique note and an early stability pilot clinical trial. Injury. 2010;41(7):693–701.
46. Zaffagnini S, Signorelli C, Lopomo N, Bonanzinga T, Marcheggiani Muccioli GM, Bignozzi S, Visani A, et al. Anatomic double-bundle and over-the-top single-bundle with additional extra-articular tenodesis: an in vivo quantitative assessment of knee laxity in two different ACL reconstructions. Knee Surg Sports Traumatol Arthrosc. 2012;20(1):153–9.