10 Tissue Engineering for the Cartilage Repair of the Ankle

Alberto Gobbi, Stefan Nehrer, Markus Neubauer, and Katarzyna Herman

10.1 Introduction

The cartilage in the ankle is a highly specialized tissue, known to be unique both in biology and anatomy, thinner than in the knee, but with a higher cell density, metabolic activity and more resistance to chronic inflammation [1]. For these reasons the ankle joint is, although often involved in sports injuries, less prone to osteoarthritic progression than other joints, and many osteochondral lesions remain clinically silent. However, larger osteochondral lesions and osteochondritis dissecans of the talus can rapidly develop unstable joint fragments, cyst formation and deterioration of the subchondral bone leading to deformation and collapse of the talus. Young, active people constitute the majority of the patients developing postresidual pain after either acute sprain or repetitive trauma that is why it is crucial that the chosen treatment method has good long-term functional outcomes. There are many treatment possibilities for osteochondral lesions (OCLs) of the talus; nevertheless a gold standard is yet to be established [2]. A systematic review by Verhagen et al. has shown that nonsurgical treatment of OLCs of the talus seems to be successful in only 45% of the cases and for that reason it is not advised [3]. Microfracture has been considered a primary line of treatment in the majority of lesions, and even though short-term results have been promising, some long-term follow-up studies have shown fair and poor results from 47.7% up to 54% [4, 5]. What is more, in our randomized study comparing microfracture, chondroplasty and osteochondral autograft transplantation, we have seen an incomplete healing on a control MRI 12 months after microfracture [6]. Ferkel et al. reported that the promising clinical outcome after microfracture deteriorated in 35% of the treated patients over a period of 5 years [7]. The primary reason of long-term failure may be the poor biomechanics and biological quality of subsequently forming fibrous cartilage, rich in type I collagen. The autologous chondrocyte implantation (ACI) was the next step in the development of osteochondral lesion treatment, and it has demonstrated good clinical outcomes [8–10]. However, the procedure has been considered demanding and required two surgeries. Evolution of tissue engineering and biomaterial science provided a substrate for the development of different scaffolds for cartilage repair. Firstly, used with chondrocytes that were seeded onto the matrix, still that did not

A. Gobbi (✉) · K. Herman
Orthopaedic Arthroscopic Surgery International (OASI), Bioresearch Foundation Gobbi NPO, Milan, Italy
e-mail: gobbi@cartilagedoctor.it

S. Nehrer · M. Neubauer
Department for Health Sciences, Medicine and Research Center for Regenerative Medicine, Center for Health Sciences and Medicine, Danube University Krems, Krems, Austria

G. L. Canata et al. (eds.), *Sports Injuries of the Foot and Ankle*,
https://doi.org/10.1007/978-3-662-58704-1_10

eliminate the need for chondrocyte harvest and cultivation. Subsequently, a need for a "biological solution to a biological problem" idea has led to the use of bone marrow aspirate concentrate (BMAC) and a hyaluronic acid-based scaffold (HA) in a one-step procedure [11].

10.2 Scaffolds for Tissue Engineering

Scaffolds are designed to host and support the cells used for cartilage regeneration. Materials used in matrices development are either naturally occurring (i.e. hyaluronan, collagen, chitosan) or synthetic (i.e. polystyrene, polylactic acid) [12]. The physical structure and the macro- and micro-architecture also vary, and liquid scaffolds entrap the cells, whereas a multilayered fibre or mesh supports implanted cells allowing their adherence [13, 14]. There are crucial criteria that characterize a good scaffold [14]. Firstly, the material must be biocompatible, and the scaffold itself and the breakdown products should not create an immune response. Secondly, the sufficient porosity of the material is important, so that it allows the cells ingrowth. Finally, the mechanical resistance to shear forces acting in the joint and scaffold stability are of great importance. Among the natural and synthetic materials that have been investigated, only a few have been used in ankle lesions.

The hyaluronan-based scaffolds are entirely based on the benzylic ester of hyaluronic acid, which is a natural glycosaminoglycan, widely distributed in connective tissues. Because of its molecular structure and multifunctional activity, it has proven to be an ideal material for tissue engineering. The network of 15–20-μm-thick fibres forms a scaffold that provides a good support that allows contact of seeded cells, subsequent cluster formation and extracellular matrix deposition. Good clinical results have been achieved in a two-step procedure using the matrix-induced autologous chondrocyte implantation technique and the use of a hyaluronic acid-based scaffold [15–17], as well as in a one-step procedure with the use of BMAC (Hyalofast, Anika Therapeutics Inc., Massachusetts, USA) [11]. Another type of scaffold used in treatment of OCL of the talus consists of collagen I and III and is a bilayer matrix that has been used in first-generation ACI and in combination with microfracture providing a good outcome [18, 19]. A scaffold used in treatment of OCL that varies in structure from collagen- and hyaluronan-based scaffolds mimics the trilateral morphology of the osteochondral unit. The superficial layer is made of type I collagen, while the lower layer consists mainly of magnesium-enriched hydroxyapatite. Although presenting clinical improvement in the treatment of OCL in the talus, it has shown limited tissue regeneration [20, 21].

10.3 Bone Marrow in Cartilage Repair

Using BMAC for cartilage regeneration is a valuable technique, offering a chance to avoid two surgeries and expensive chondrocyte cultivation. BMAC has proven to be a good material for cell-based therapy in cartilage regeneration with a potential to differentiate into osteogenic and chondrogenic cells [22–24]. Moreover, many studies and publications have proven that BMAC has the ability to restore healthy and functional tissues even in cases of high-grade articular cartilage injury [11, 25–27]. The bone marrow aspirate (BMA) is usually harvested from an ipsilateral iliac crest prior to the main procedure. A sharp trocar with an aspiration needle is placed in the bone between the cortices, about 3–5 cm deep. An average total aspiration volume of 60 mL is harvested, using a standard syringe. Frequently used centrifugation systems include the "RegenKit Extracell BMC" (Regen Lab, Le Mont-sur-Lausanne, Switzerland), "Arthrex Angel®"(Arthrex, Naples, United States), "Harvest Technologies system" (Plymouth, MA) or the "Cobe 2991 Cell Processor" (Terumo BCT, Paris, France) [28].

The aspirate is then prepared and centrifuged to obtain a concentrated product. The rationale behind the process is to increase the proportion of mesenchymal stem/progenitor cells (MSCs) in plain BMA, which is in between 0.001 and 0.01% of the nucleated cells [29]. The process of centrifugation not only results in a higher proportion of MSCs but also higher concentration of platelets and disrupts cell components increasing free

growth factors that might be predominantly relevant for the regenerative processes. The average processing time takes around 15 min, but a newly introduced bone marrow retrieval system (Marrow Cellution®) may reduce time and cost of the procedure and avoid regulation problems regarding cell manipulation by centrifugation. Combination of gradual aspiration through a system of lateral holes reduces the peripheral blood harvest, which results in an aspirate consisting a greater amount of fibroblast-like colony-forming units (CFU-f) without the centrifugation step.

10.4 Scaffold and Stem Cell Surgical Technique

The first and crucial decision in the surgical treatment of OCL of the talus is if the defect is accessible through an anterior approach or a medial malleolar osteotomy is needed in case of the medial talar dome OCL. Lesions on the lateral side are usually more accessible in plantar flexion and only in rare cases require a fibular osteotomy, which is a technically challenging procedure. Figure 10.1 shows basic surgical procedures to access chondral lesions of the talus [30]. The second decision is if an osseous reconstruction is necessary in addition to the cartilage repair procedure. In that case, cancellous bone can be harvested from the tibia or from the iliac crest with a coring drill instrument to provide a stable bony reconstruction [31]. Defects that are deeper than 5 mm are considered indicated for cancellous bone filling as has been stated in the latest published recommendations of a consensus group [32]. For chondral defects without bony defect, the same group also recommended the use of a biomaterial to facilitate cartilage tissue formation and support fill of the defect, especially in defect sizes bigger than 10 mm in diameter. The treatment options are the application of a biomaterial, mostly hyaluronan-based scaffold, filled with bone marrow aspirate concentrate (BMAC) preferable without microfracture. The bone marrow harvested from the iliac crest is a source of cells that provide a biological regenerative potential in the defect without disturbing the subchondral bone. However, a thorough debridement of the defect and removal of any unstable fragments in the cartilage or bone is mandatory for a successful outcome. The surgical application technique requires bone marrow aspiration followed by its concentration, as well as the seeding of the scaffold and the implantation procedure. Trials investigating BMAC in combination with scaffolds used this approach for type II chronic talus cartilage lesions of >1.5 cm^2 [22, 33].

Firstly, bone marrow is harvested and centrifuged to obtain a concentrated product (Fig. 10.2a). We advocate the use of batroxobin enzyme (Plateltex Act, Plateltex SRO, Bratislava, Slovakia), to activate BMAC and to produce a sticky clot material (Fig. 10.2b) that makes the application into the defect easier. A standard

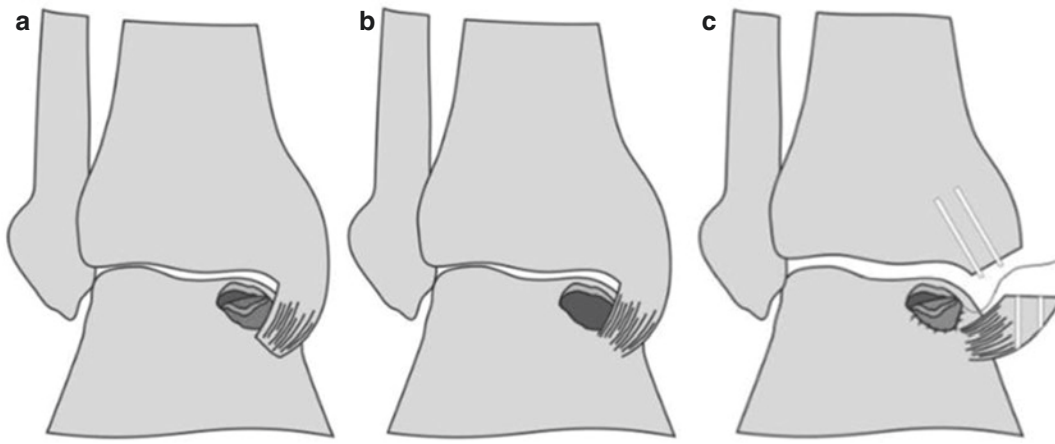

Fig. 10.1 Surgical procedures to access osteochondral lesions of the talus (**a**) delaminated piece of cartilage, (**b**) debrided defect and (**c**) malleolar osteotomy and suturing

ankle arthroscopic procedure is performed, and the lesion site is visualized (Fig. 10.3a), debrided until healthy bone (Fig. 10.3b) and clear cartilage edges are visible and measured. According to the measurements, a scaffold is cut to fit into the defect side. For a 2 × 2 cm hyaluronan scaffold,

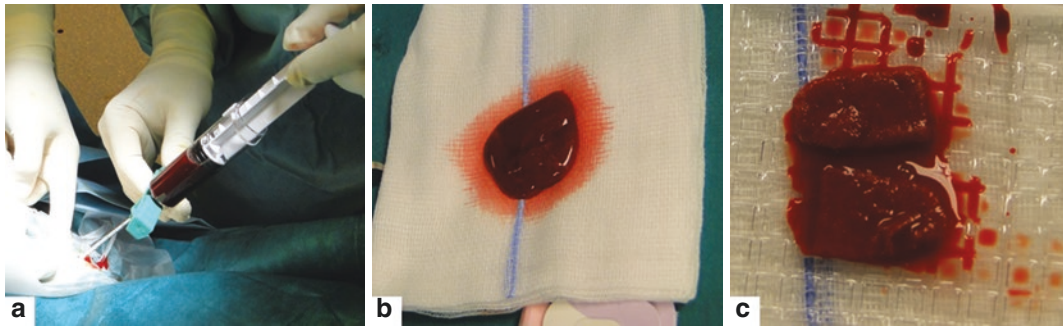

Fig. 10.2 Preparation of the HA-BMAC. (**a**) Harvesting bone marrow from the ipsilateral iliac crest using a sharp trocar (**b**) bone marrow aspirate concentrate (BMAC) after activation with batroxobin enzyme forms a sticky clot (**c**) hyaluronic acid-based (HA) scaffold combined with BMAC clot ready for implantation

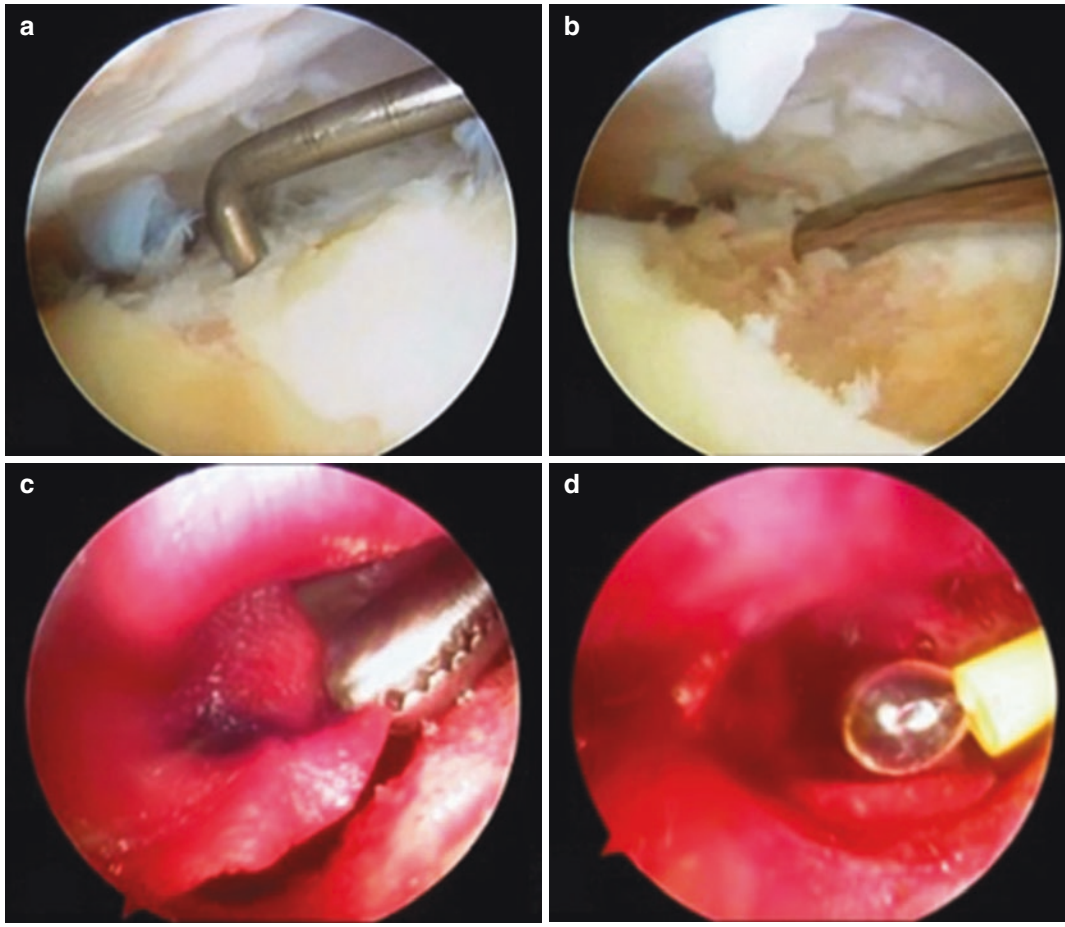

Fig. 10.3 Arthroscopic procedure with the use of HA-BMAC osteochondral lesion of the talus. (**a**) Identification of the lesion on the talar dome, (**b**) lesion debridement with a curette, (**c**) placement of the HA-BMAC into the lesion and (**d**) adding fibrin glue to secure the scaffold

approximately 2–3 mL of BMAC is needed to seed the matrix (Fig. 10.2c). The seeded matrix is then placed onto the debrided or bone augmented defect side (Fig. 10.3c). It is recommended to use a cannula or halfpipe-like instrument in order to safely transport the matrix into the joint. This surgical step might be challenging, sometimes a slight widening of the arthroscopic approach is necessary, but special devices have been designed to aid this crucial step. After scaffold placement some authors add platelet-rich plasma or platelet-rich fibrin (Fig. 10.3d).

Alternatively, in cases of bigger defects or problems with the arthroscopic technique, the scaffold can be properly placed using an open approach. Finally, the ankle is moved under visual control to ensure the correct placement and stability of the implanted scaffold. In cases of malleolar osteotomy, the bone fragment is reduced and fixed with screws; the holes for screw placement should be predrilled before the osteotomy to achieve a full anatomical reconstruction.

10.5 Conclusion

For treatment of osteochondral lesions of the talus, the addition of biologics, primarily BMAC, is recommended by the evidence level C studies. Giannini et al. showed significant improvements in AOFAS score and histological and immunohistochemical appearance up to 24 months post-treatment [33]; in a follow-up trial, the AOFAS score decreased at 36 and 48 months post-treatment and plateaued at 72 months [22]. Vannini et al. presented another insightful result; the authors could show that around 97% of patients could return to activity and 73% returned to sports at a preinjury level [34]. Based on the current evidence, the use of biomaterial and biological augmentation with BMAC can be used in the treatment of osteochondral lesions of the talus. Nevertheless, more long-term results are needed to fortify these recommendations.

References

1. Shepherd DET, Seedhom BB. Thickness of human articular cartilage in joints of the lower limb. Ann Rheum Dis. 1999;58(1):27–34.
2. Murawski CD, Kennedy JG. Operative treatment of osteochondral lesions of the talus. J Bone Joint Surg Am. 2013;95(11):1045–54.
3. Verhagen RA, Struijs PA, Bossuyt PM, van Dijk CN. Systematic review of treatment strategies for osteochondral defects of the talar dome. Foot Ankle Clin. 2003;8(2):233–42.
4. Hunt SA, Sherman O. Arthroscopic treatment of osteochondral lesions of the talus with correlation of outcome scoring systems. Arthroscopy. 2003;19(4):360–7.
5. Robinson DE, Winson IG, Harries WJ, Kelly AJ. Arthroscopic treatment of osteochondral lesions of the talus. J Bone Joint Surg (Br). 2003;85(7):989–93.
6. Gobbi A, Francisco RA, Lubowitz JH, Allegra F, Canata G. Osteochondral lesions of the talus: randomized controlled trial comparing chondroplasty, microfracture, and osteochondral autograft transplantation. Arthroscopy. 2006;22(10):1085–92.
7. Ferkel RD, Zanotti RM, Komenda GA, Sgaglione NA, Cheng MS, Applegate GR, Dopirak RM. Arthroscopic treatment of chronic osteochondral lesions of the talus: long-term results. Am J Sports Med. 2008;36(9):1750–62.
8. Giannini S, Battaglia M, Buda R, Cavallo M, Ruffilli A, Vannini F. Surgical treatment of osteochondral lesions of the talus by open-field autologous chondrocyte implantation: a 10-year follow-up clinical and magnetic resonance imaging T2-mapping evaluation. Am J Sports Med. 2009;37(Suppl 1):112S–8S.
9. Kwak SK, Kern BS, Ferkel RD, Chan KW, Kasraeian S, Applegate GR. Autologous chondrocyte implantation of the ankle: 2- to 10-year results. Am J Sports Med. 2014;42(9):2156–64.
10. Pereterson L, Mandelbaum B, Gobbi A, Francisco R, Autologous Chondrocyte transplantation of the ankle, Basic science, clinical repair and reconstruction of articular cartilage defects: current status and prospects. Timeo. 2006:341–347.
11. Gobbi A, Karnatzikos G, Sankineani SR. One-step surgery with multipotent stem cells for the treatment of large full-thickness chondral defects of the knee. Am J Sports Med. 2014;42(3):648–57.
12. O'Brien F. Biomaterials & scaffolds for tissue engineering. Mater Today. 2011;14(3):88–95.
13. Scotti C, Leumann A, Candrian C, et al. Autologous tissue-engineered osteochondral graft for talus osteochondral lesions: state-of-the-art and future perspectives. Tech Foot & Ankle Surg. 2011;10(4):163–8.
14. Frenkel S, Di Cesare P. Scaffolds for articular cartilage repair. Ann Biomed Eng. 2004;32(1):26–34.

15. Marcacci M, Berruto M, Brocchetta D, et al. Articular cartilage engineering with Hyalograft C: 3-year clinical results. Clin Orthop Relat Res. 2005;435:96–105.
16. Gobbi A, Kon E, Berruto M, et al. Patellofemoral full-thickness chondral defects treated with Hyalo-graft-C: a clinical, arthroscopic, and histologic review. Am J Sports Med. 2006;34:1763–73.
17. Gobbi A, Katzarnikos G, Lad D. Osteochondral lesions of the talar dome: matrix-induced autologous chondrocyte implantation. In: The foot and ankle: AANA advanced arthroscopic surgical techniques. Thorofare: Slack Inc; 2016. p. 37–48.
18. McCarthy HS, Roberts S. A histological comparison of the repair tissue formed when using either Chondrogide(®) or periosteum during autologous chondrocyte implantation. Osteoarthr Cartil. 2013;12:2048–57.
19. Valderrabano V, Miska M, Leumann A, et al. Reconstruction of osteochondral lesions of the talus with autologous spongiosa grafts and autologous matrix-induced chondrogenesis. Am J Sports Med. 2013;41(3):519–27.
20. Albano D, Martinelli N, Bianchi A, Messina C, Malerba F, Sconfienza LM. Clinical and imaging outcome of osteochondral lesions of the talus treated using autologous matrix-induced chondrogenesis technique with a biomimetic scaffold. BMC Musculoskelet Disord. 2017;18(1):306.
21. 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):2380–7.
22. Giannini S, Buda R, Battaglia M, et al. One-step repair in talar osteochondral lesions:4-year clinical results and t2-mapping capability in outcome prediction. Am J Sports Med. 2013;41:511–8.
23. Cavallo C, Desando G, Cattini L, et al. Bone marrow concentrated cell transplantation: rationale for its use in the treatment of human osteochondral lesions. J Biol Regul Homeost Agents. 2013;27(1):165–75.
24. Mesenchymal CA. Stem cells. The past, the present, the future. Cartilage. 2010;1(1):6–9.
25. Buda R, Vannini F, Castagnini F, et al. Regenerative treatment in osteochondral lesions of the talus: autologous chondrocyte implantation versus one-step bone marrow derived cells transplantation. Int Orthop. 2015;39:893–900.
26. Gobbi A, Karnatzikos G, Scotti C, et al. One-step cartilage repair with bone marrow aspirate concentrated cells and collagen matrix in full-thickness knee cartilage lesions: results at 2-year follow-up. Cartilage. 2011;2(3):286–99.
27. Gobbi A, Whyte GP. Osteochondritis dissecans: pathoanatomy, classification, and advances in biologic surgical treatment. In: Bio-orthopedics. Berlin, Heidelberg: Springer; 2017. p. 489–501.
28. Murray IR, Robinson PG, West CC, et al. Reporting standards in clinical studies evaluating bone marrow aspirate concentrate: a systematic review. Arthrosc J Arthrosc Relat Surg. 2018;34(4):1366–75.
29. Kasten P, Beyen I, Egermann M, et al. Instant stem cell therapy: characterization and concentration of human mesenchymal stem cells in vitro. Eur Cell Mater. 2008;16:47–55.
30. Nehrer S, Domayer SE, Hirschfeld C, Stelzeneder D, Trattnig S, Dorotka R. Matrix-associated and autologous chondrocyte transplantation in the ankle: clinical and MRI follow-up after 2 to 11 years. Cartilage. 2011;2(1):81.
31. Sadlik B, Gobbi A, Puszkarz M, Klon W, Whyte GP. Biologic inlay osteochondral reconstruction: arthroscopic one-step osteochondral lesion repair in the knee using morselized bone grafting and hyaluronic acid-based scaffold embedded with bone marrow aspirate concentrate. Arthrosc Tech. 2017;6(2):e383.
32. Rothrauff BB, Murawski CD, Angthong C, et al. Scaffold-based therapies: proceedings of the international consensus meeting on cartilage repair of the ankle. Foot Ankle Int. 2018;39:41S–7S.
33. Giannini S, Buda R, Vannini F, Cavallo M, Grigolo B. One-step bone marrow-derived cell transplantation in talar osteochondral lesions. Clin Orthop Relat Res. 2009;467(12):3307–20.
34. Vannini F, Cavallo M, Ramponi L, et al. Return to sports after bone marrow–derived cell transplantation for osteochondral lesions of the talus. Cartilage. 2017;8(1):80–7.