Chapter 6
Hyaluronic Acid

Hélder Pereira, Duarte Andre Sousa, António Cunha, Renato Andrade, J. Espregueira-Mendes, J. Miguel Oliveira, and Rui L. Reis

Abstract In recent times, the field of tissue engineering and regenerative medicine (TERM) has considerably increased the extent of therapeutic strategies for clinical application in orthopedics. However, TERM approaches have its rules and requirements, in the respect of the biologic response of each tissue and bioactive agents which need to be considered, respected, and subject of ongoing studies. Different medical devices/products have been prematurely available on the market and used in clinics with limited success. However, other therapeutics, when used in a serious and evidence-based approach, have achieved considerable success, considering the respect for solid expectations from doctors and patients (when properly informed).

H. Pereira (✉)
3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga, Portugal

Orthopedic Department of Póvoa de Varzim – Vila do Conde Hospital Centre, Póvoa de Varzim, Portugal

Ripoll y De Prado Sports Clinic: Murcia-Madrid FIFA Medical Centre of Excellence, Murcia/Madrid, Spain

International Centre of Sports Traumatology of the Ave, Taipas, Portugal
e-mail: helderduartepereira@gmail.com

D. A. Sousa
Orthopedic Department of Póvoa de Varzim – Vila do Conde Hospital Centre, Póvoa de Varzim, Portugal

A. Cunha
International Centre of Sports Traumatology of the Ave, Taipas, Portugal

R. Andrade
Clínica do Dragão, Espregueira-Mendes Sports Centre – FIFA Medical Centre of Excellence, Porto, Portugal

J. M. Oliveira et al. (eds.), *Osteochondral Tissue Engineering*,
Advances in Experimental Medicine and Biology 1059,
https://doi.org/10.1007/978-3-319-76735-2_6

Orthobiologics has appeared as a recent technological trend in orthopedics. This includes the improvement or regeneration of different musculoskeletal tissues by means of using biomaterials (e.g., hyaluronic acid), stem cells, and growth factors (e.g., platelet-rich plasma). The potential symbiotic relationship between biologic therapies and surgery makes these strategies suitable to be used in one single intervention.

However, herein, the recent clinical studies using hyaluronic acid (HA) in the treatment of orthopedic conditions will mainly be overviewed (e.g., osteochondral lesions, tendinopathies). The possibilities to combine different orthobiologic agents as TERM clinical strategies for treatment of orthopedic problems will also be briefly discussed.

Keywords Osteochondral lesions · Tendinopathies · Orthobiologics · Hyaluronic acid · Stem cells · Platelet-rich plasma · Growth factors · Tissue engineering and regenerative medicine

---

J. Espregueira-Mendes
3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga, Portugal

Clínica do Dragão, Espregueira-Mendes Sports Centre – FIFA Medical Centre of Excellence, Porto, Portugal

Dom Henrique Research Centre, Porto, Portugal

Orthopedic Department, University of Minho, Braga, Portugal

J. M. Oliveira
3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga, Portugal

Ripoll y De Prado Sports Clinic: Murcia-Madrid FIFA Medical Centre of Excellence, Murcia/Madrid, Spain

The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

R. L. Reis
3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Barco, Guimarães, Portugal

ICVS/3B's – PT Government Associated Laboratory, Braga, Portugal

The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Guimarães, Portugal

Top 10 Evidence-Based References

1. Gigante A, Callegari L (2011) The role of intra-articular hyaluronan (Sinovial) in the treatment of osteoarthritis. Rheumatol Int 31(4):427–444. https://doi.org/10.1007/s00296-010-1660-6
2. Strauss EJ, Hart JA, Miller MD, Altman RD, Rosen JE (2009) Hyaluronic acid viscosupplementation and osteoarthritis: current uses and future directions. Am J Sports Med 37(8):1636–1644. https://doi.org/10.1177/0363546508326984
3. Xing D, Wang B, Liu Q, Ke Y, Xu Y, Li Z, Lin J (2016) Intra-articular hyaluronic acid in treating knee osteoarthritis: a PRISMA-compliant systematic review of overlapping meta-analysis. Sci Rep 6:32790. https://doi.org/10.1038/srep32790
4. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G (2006) Viscosupplementation for the treatment of osteoarthritis of the knee. Cochrane Database Syst Rev (2):CD005321. https://doi.org/10.1002/14651858.CD005321.pub2
5. Witteveen AG, Hofstad CJ, Kerkhoffs GM (2015) Hyaluronic acid and other conservative treatment options for osteoarthritis of the ankle. Cochrane Database Syst Rev (10):CD010643. https://doi.org/10.1002/14651858.CD010643.pub2
6. Colen S, Geervliet P, Haverkamp D, Van Den Bekerom MP (2014) Intra-articular infiltration therapy for patients with glenohumeral osteoarthritis: a systematic review of the literature. Int J Shoulder Surg 8(4):114–121. https://doi.org/10.4103/0973-6042.145252
7. Trellu S, Dadoun S, Berenbaum F, Fautrel B, Gossec L (2015) Intra-articular injections in thumb osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. Joint Bone Spine 82(5):315–319. https://doi.org/10.1016/j.jbspin.2015.02.002
8. Lynen N, De Vroey T, Spiegel I, Van Ongeval F, Hendrickx NJ, Stassijns G (2017) Comparison of peritendinous hyaluronan injections versus extracorporeal shock wave therapy in the treatment of painful achilles' tendinopathy: a randomized clinical efficacy and safety study. Arch Phys Med Rehabil 98(1):64–71. https://doi.org/10.1016/j.apmr.2016.08.470
9. Kumai T, Muneta T, Tsuchiya A, Shiraishi M, Ishizaki Y, Sugimoto K, Samoto N, Isomoto S, Tanaka Y, Takakura Y (2014) The short-term effect after a single injection of high-molecular-weight hyaluronic acid in patients with enthesopathies (lateral epicondylitis, patellar tendinopathy, insertional Achilles tendinopathy, and plantar fasciitis): a preliminary study. J Orthop Sci 19 (4):603–611. https://doi.org/10.1007/s00776-014-0579-2

10. Tsaryk R, Gloria A, Russo T, Anspach L, De Santis R, Ghanaati S, Unger RE, Ambrosio L, Kirkpatrick CJ (2015) Collagen-low molecular weight hyaluronic acid semi-interpenetrating network loaded with gelatin microspheres for cell and growth factor delivery for nucleus pulposus regeneration. Acta Biomater 20:10–21. https://doi.org/10.1016/j.actbio.2015.03.041

Fact Box 1 – What Is Hyaluronic Acid

- Hyaluronic acid (HA) is a high molecular weight biopolysaccharide, discovered in 1934.
- HA is a naturally occurring biopolymer, found in most connective tissues, and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, umbilical cords, and chicken combs.
- It is a high viscoelastic fluid capable to reproduce and repair the rheological proprieties of the synovial fluid.
- Besides its rheological proprieties, it acts as a shock absorber and as a lubricant and has anti-angiogenic, anti-inflammatory, and analgesic properties as well as immunosuppressive capacities.
- As a hydrogel or tridimensional scaffold, it can be used as protein or cell carrier.

Fact Box 2 – Clinical Experience with Hyaluronic Acid in Orthopedics

- Hyaluronic acid (HA) has been used mostly in the treatment of cartilage and osteoarthritis.
- It has been used in several joints (knee, ankle, shoulder, hip, first carpometacarpal, etc.).
- It has shown transient benefits in pain relief and improved range of motion (minimum 6 months).
- It has rare adverse effects (mainly self-limited pain and swelling (spontaneously solved within 48 h)).
- It has shown promising results in the treatment of tendinopathies including enthesopathies.
- Intra-tendon injections might have deleterious effects.

Fact Box 3 – Hyaluronic Acid and Tissue Engineering and Regenerative Medicine

- Tissue engineering and regenerative medicine (TERM) aims for more advanced approaches of tissue regeneration and disease control (combining scaffolds, cells, growth factors, prolotherapy, nanotechnology, bioreactors, gene therapy, etc.).
- Advanced TERM approaches using hyaluronic acid (HA) have been tested including combination of HA with growth factors, cells, nanotechnology, and advanced scaffolds.
- Dealing with cartilage (including hyaline or fibrocartilage) and tendon regeneration represents two of the most challenging tissues in the field of clinical orthopedics, and the road for the future for sure will comprise advanced TERM approach.
- The achieved knowledge from the clinical experience with HA must be used as a launching platform for future basic science studies. Furthermore, higher-quality clinical studies are required for more accurate conclusions.

6.1 Introduction

The treatment of osteochondral (OC) defects and/or osteoarthritis in different joints remains a challenge, and the quest for the optimal conservative treatment continues. This represents an important socioeconomic burden given the high prevalence of these diseases and possibilities to cause functional impairment. In epidemiology, half of the world's population aged 65 years or older has osteoarthritis (OA), which is the most prevalent disorder of articulating joints in humans [1]. The estimated social cost of OA might range between 0.25% and 0.50% of a country's gross domestic product [2]. Degeneration related to the aging process, trauma-related injuries, and degenerative or idiopathic disorders can lead to OC lesions [3]. A distinction should be made between the chondral lesion in which the damage occurs on chondrocytes and articular cartilage extracellular matrix (ECM) opposing to OC defects in which, besides cartilage damage, the subchondral bone tissue is also affected [4]. The hyaline cartilage layer has highly flexible and supportive characteristics. The unique features of the cartilage tissue limit its regenerative capacity due to the lack of vascularization and innervation [5]. Similarly, cartilage has very limited remodeling possibilities given the low number of cells and low metabolic activity of chondrocytes (mainly the mature ones) which produces less extracellular matrix (ECM) [6, 7].

Damage to articular cartilage preceded by joint trauma is a major risk factor leading to progression of OA [8]. However, OA has a higher incidence in aged people and it is strongly correlated with natural aging process [9]. The huge variability of the OA tissues between individuals is proven to be one of the most important factors affecting the understanding of the disease and the variability in

response to therapeutics. The current treatments are based on the adaptation of lifestyle and on the use of anti-inflammatory and painkiller drugs or clinically solved with the substitution by an artificial implant.

Another very important field within orthopedics is related to tendinopathies within its many presentation forms which affect a high number of patients [10, 11]. Tendon is also a tissue with low mitotic activity and with limited self-repair capacity [12].

Currently, there has been growing popularity of non-operative therapies, which are able to induce the body's self-repair and recovery from injuries or simply improve the symptoms without relevant secondary effects. These therapies include hyaluronic acid injections which are included in a new therapeutic field named orthobiologics [13]. Despite hyaluronic acid, orthobiologics also enrolls the clinical use of growth factors (e.g., platelet-rich plasma, bone morphogenetic proteins) or mesenchymal stem cells [14–21].

Viscosupplementation, which concerns to hyaluronic acid injections, was the first generation of orthobiologics. The treatment was firstly done in 1997 to relieve patients from pain symptoms of OA [1]. The outcome was satisfactory, and when compared to oral drug administration (NSAID), viscosupplementation was able to diminish patient's pain.

Herein only the clinical use and potential future applications of hyaluronic acid will be presented. Most studies related to orthobiologics have several methodological limitations. Despite the growing evidence supporting some therapeutic strategies, more high-level studies with uniform outcome measures are required in order to assess the present and prepare the future.

6.2 What Is Hyaluronic Acid?

Hyaluronic acid (HA) injection (Fig. 6.1) at the injury site has been used as a conservative method to treat OC lesions, osteoarthritis, and tendinopathies [1, 14, 16, 22–28]. In brief, HA is a high molecular weight biopolysaccharide, discovered in

Fig. 6.1 Commercial formulation of hyaluronic acid injection ready for clinical use under strict aseptic conditions

1934, by Karl Meyer and John Palmer in the vitreous of bovine eyes [29]. HA is a naturally occurring biopolymer, which has important biological functions in bacteria and higher animals including humans. It is found in most connective tissues and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, umbilical cords, and chicken combs [29]. It is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases and degraded by a family of enzymes called hyaluronidases. The first medical HA application in humans was in the vitreous substitution/replacement during eye surgery, in the late 1950s [22, 29]. Viscosupplementation (VS) came into clinical use in Japan and Italy in 1987 and in Canada in 1992 but was adopted in Europe and the USA in the second half of the 1990s [29].

VS can be described as the intra-articular administration of a high viscoelastic fluid into the synovial joint to reproduce and repair the rheological proprieties of the synovial fluid. VS can enhance the vital joint lubrication and shock absorption ability, essential functions for mobility improvements, and pain relief. All market available products for VS are based on hyaluronic acid (HA), a high molecular weight (105–107 Da) and unbranched glycosaminoglycan that can be found in the extracellular matrix of human tissue.

Despite HA being described as safe and effective, several of its functions within the body remain unknown [30, 31]. HA has shown chondroprotective effects in vivo and in vitro [29]. Combined with its rheological proprieties, this helps to explain the beneficial long-term effects on articular cartilage. HA also reduces pain-associated nerve impulses and sensitivity. HA is a free, non-sulfated, and negatively charged glycosaminoglycan (GAG) capable of interacting with receptors and ECM proteins [32].

HA can be derived from different sources such as rooster combs, bacterial production, or either animal or human sources [33]. Its properties (e.g., rheological properties) depend on its source. Nevertheless, HA solutions always present high viscosity. It is a water-soluble polymer and has specific enzymatic degradation. There are two forms of HA, based in the chain length: low and high molecular weight ($\leq 2 \times 10^6$ Da and $2 \times 10^6 - \geq 4 \times 10^6$ Da, respectively). Structural and biological functions of HA vary among these different presentations as suggested by Stern et al. [34, 35]. The interactions between tissues and HA occur through hyaladherins. Functions such as cell communication, motility, and morphogenesis occur due to interactions between hyaladherins and tissue receptors, mainly CD44 and RHAMM at the cell surface [29]. The hyaluronic acid high molecular weight (HMWHA) molecule plays a structural role by being able to bind 10 to 10,000 time its weight in water [34, 36, 37]. Thus, osmotically active in a completely hydrated state, it is able to fill the space acting as a shock absorber and also as a lubricant. From a biological point of view, the HMW chains are anti-angiogenic and anti-inflammatory and possess immunosuppressive capacities [38, 39]. Wide ranges of studies have reported a decrease in the inflammatory response and apoptosis through the downregulation of many factors responsible for ECM. These results suggest that HMWHA impairs the phenomena of phagocytosis, macrophage activation, and inflammatory cytokines production. This can also explain its possible beneficial effects in tendinopathies.

However, HMWHA chains can break down into low molecular weight chains (LMWHA), which are found to have a pro-inflammatory effect [26]. These fragments have been shown to secrete inflammatory cytokines and stimulate angiogenesis and tissue remodeling after activation of endogenous signaling pathways. They can promote the activation and maturation of dendritic cells and release of pro-inflammatory cytokines [35, 39, 40]. Molecular changes in the ECM of damaged joints alter the composition and structure of natural HA. Along with molecule secretion and tissue remodeling, the development of pathologies also occurs [34]. So, a lot remains to understand in order to improve efficacy of this therapeutic agent.

6.3 Most Frequent Isolated Injection Therapy in Different Joints and Tendons

HA injection, also known as viscosupplementation (VS), constitutes a conservative treatment to improve the biomechanical function of the joint and/or tendons mainly due to HA physicochemical characteristics (i.e., hydrogel state) [41, 42]. HA is a gel-like constituent injected in the joint or tendon sheath [1, 43]. In some cases, to ensure safety or effectiveness of HA delivery, its application can be guided by ultrasound or X-ray fluoroscopy [44]. It is thought that HA acts as lubricant displaying a cushion effect. However, the biological mechanism behind this role in cartilage/tendon repair still remains a debate in medical community [45].

6.3.1 Intra-Articular Application

Positive effects of HA intra-articular injections have been reported in the conservative treatment of symptomatic osteoarthritis [46, 47]. It can be used as alternative to surgical approach mainly in the early phases of osteoarthritis or in patients without medical conditions to undergo more aggressive surgical treatment (e.g., replacement arthroplasty) [1, 27, 31, 48–51]. Viscosupplementation can be performed as augmentation after surgical intervention to treat osteochondral lesions [52]. The Food and Drug Administration (FDA) regulates the use of HA for intra-articular injection. Several forms of HA have received FDA approval for clinical use [53], despite the biological mechanism of HA is not fully explained as well as the differences between the several HA presentations. There is still debate and controversy in literature concerning the best approach for each joint and grade of disease [54].

HA has a half-life of less than 24 h after intra-articular injection [29]. Short half-life, due to the rapid breakdown and reabsorption of HA, might represent a limitation in the intra-articular injections. For this reason, sustained-release approaches are under development. It has been already suggested in clinical trials that combination of treatments is shown to be more effective than HA alone [55–60].

The highest clinical experience available is related to knee (Fig. 6.2) and ankle (Fig. 6.3) use of HA [1, 31]. Protocols range from one single shot treatment to treatments requiring three to five injections, depending on the formulation of HA

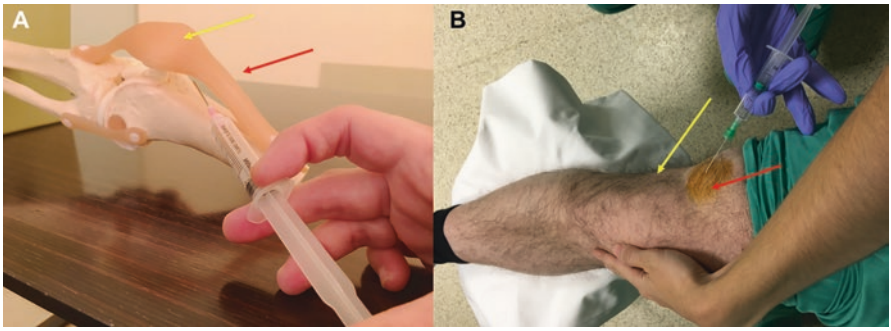

Fig. 6.2 A schematic representation of the knee injection of hyaluronic acid. (**A**) Model where the patella is visible (yellow arrow) which must be relaxed; the needle should be introduced in the lateral suprapatellar pouch (red arrow) on an oblique orientation toward the joint. Usually, an experienced operator is capable to sense the exact moment when he trespasses the joint capsule with the needle. After this, the gel can be introduced safely. (**B**) Clinical representation where the patella (yellow arrow) and the suprapatellar pouch (red arrow) are visible. The operator slightly moves the patella laterally to relax the suprapatellar pouch

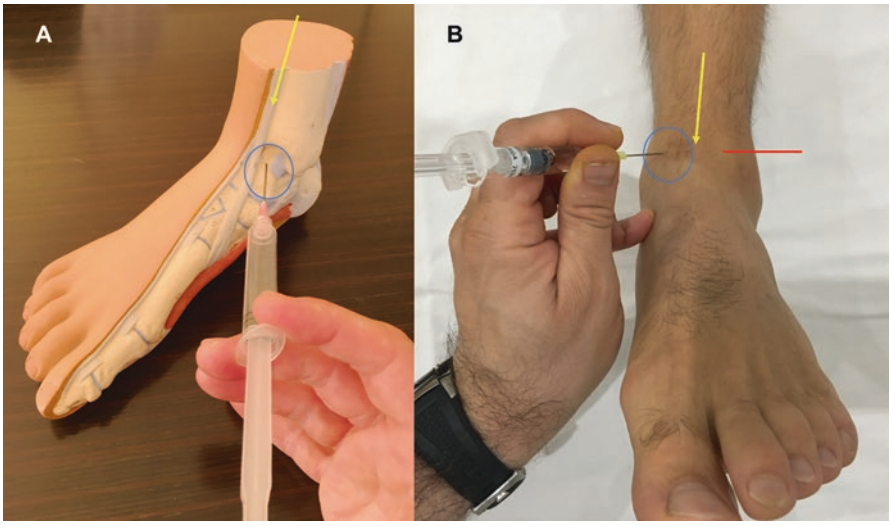

Fig. 6.3 A schematic representation of ankle injection of hyaluronic acid (**A**). The anterior tibial tendon (ATT) is visible (yellow arrow). The injection is given with the ankle in dorsiflexion, which dislocates the ATT centrally, in the soft spot where you can feel the joint line (blue circle). Again, an experienced operator is capable to sense the exact moment when he trespasses the joint capsule with the needle. After this, the gel can be introduced safely. (**B**) Clinical representation of the ankle injection. Yellow arrow represents the ATT, red line represents the joint line, and the little finger palpates the tip of the medial malleolus (usually the joint line will be 1.5–2 cm above)

and the condition of the patient [26]. The possible adverse reactions are rare (1–2% of cases) and consist usually in transient pain and/or swelling, which usually resolve spontaneously in 48 h [26]. No serious adverse effects have been reported.

A recent meta-analysis of all high-quality previously published meta-analysis on the subject of HA use in the knee joint by Xing et al. showed that HA provided a moderate but real benefit for patients with knee OA [1]. This study, a systematic review of overlapping meta-analyses, that investigated efficacy and safety of HA, in treating knee OA, concluded that currently, the best evidence suggested that HA is an effective intervention in treating knee OA without increased risk of adverse events. It concluded that US-approved HA is safe and efficacious through an average of 26 weeks in treating symptomatic knee OA.

Therefore, the evidence supports the use of the HA in the treating knee OA. Further studies with effect size statistic are still required to qualify the clinical efficacy [1].

Concerning the ankle, a recent Cochrane review by Witteveen et al. identified six randomized controlled studies on the topic and concluded that it remains unclear which patients (age, grade of ankle OA) benefit the most from HA injections and which dosage schedule should be used [31]. Considering the best available evidence, HA can be conditionally recommended if patients have an inadequate response to simple analgesics. Moreover, the authors highlight the study limitations due to methodological issues of the published studies [31]. Some authors highlight the difference in congruency when comparing the ankle (congruent) joint to the knee (incongruent) joint [4]. If that would be the case, theoretically, the rheological properties of HA should be more prone to work on the ankle when compared to the knee.

More recently, several studies have stated the benefits of HA application in the shoulder. The shoulder is the most "unstable" joint of the body given its wide range of motion (including circumferential motion). On a recent study enrolling 41 patients suffering from chronic shoulder pain with limitation of motion due to glenohumeral joint OA, the authors concluded that HA may be a safe and effective treatment option for pain and stiffness and that the effects of the injections are still present for up to 6 months after the treatment [51]. However, a systematic review on the topic failed to provide definitive indications once more for methodological limitations of available literature [61]. However, HA was shown to provide consecutively better outcomes when compared to placebo.

Hip OA has also been approached, either in severe cases or in young patients (in which there is advantage to delay arthroplasty replacement) [27, 44, 49]. In the hip, several authors advise for the benefits of ultrasound-assisted injection (Fig. 6.4) (particularly in more severe OA which makes it technically much more difficult to do blindly) [44]. The preliminary results have been positive in diminishing pain and improving mobility for a minimum period of 6 months.

HA has also been tried in smaller joints like the first carpometacarpal joint of the thumb [50]. From a recent meta-analysis, it seems that the most symptomatic patients are the most prone for improvement and, when compared to corticosteroid, the positive results stand for a longer period [62].

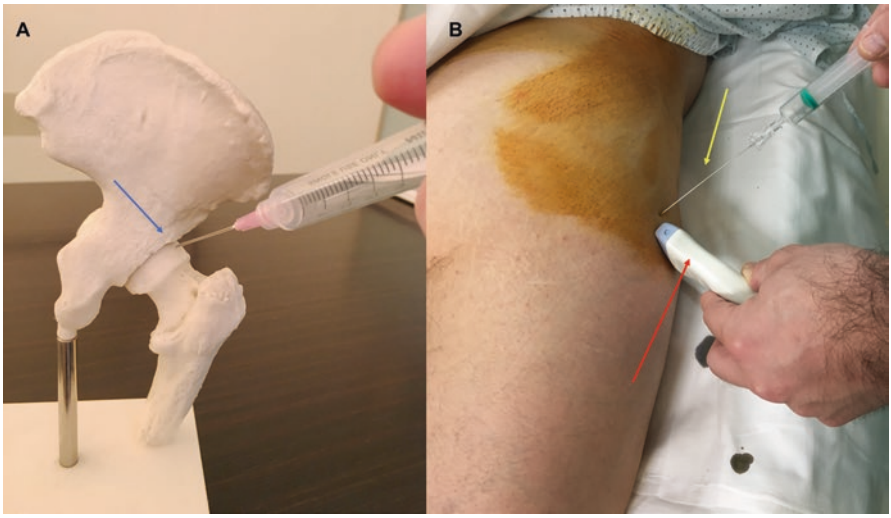

Fig. 6.4 A schematic representation of injection of hyaluronic acid in the hip joint (blue line) (**A**). (**B**) Clinical representation of hip joint injection assisted by ultrasound (red arrow), with progressive introduction of the needle (yellow arrow)

6.3.2 Clinical Use in Tendinopathies

Tendinopathies, in its many presentations, represent a current challenge in orthopedics, and in many cases, surgical options provide poor outcome [10, 11, 63]. As aforementioned, the tendon has reduced self-repair capacity, poor vascularity, and low mitotic activity [12, 63].

Promising results have been achieved in the rotator cuff with HA injection treatment. A multicenter, randomized, controlled trial assessing peritendinous HA outcome in patients with supraspinatus tendinopathy reported improved outcome and faster rehabilitation with lower number of physiotherapy sessions [25]. Also in non-calcific rotator cuff tendinopathies, when comparing HA to extracorporeal shockwaves therapy (ESWT), injections of HA provided faster clinical improvement compared to ESWT, which might result in more gradual improvement over time [64].

Some promising results have also been achieved in a preliminary study on enthesopathies (lateral epicondylitis, patellar tendinopathy, insertional Achilles tendinopathy, and plantar fasciitis) [65]. However, further research is required.

Concerning the Achilles tendon, a recent randomized study comparing peritendinous HA injections with standard ESWT has shown better outcome with HA [43]. However, one anima-based study suggests that intra-tendon HA injection might have deleterious effect and should be avoided [66]. Once more, more evidence is required.

6.4 Can Hyaluronic Acid Be Combined with Other Orthobiologic Therapies?

Not only basic science research but also some preliminary clinical data suggest that HA can be combined with other orthobiologics (growth factors, cells, biomaterials, hydrogels/scaffolds) but further suggest that there might be several advantages in doing it [67–69]. However, doing it properly and adequately, the rules of TERM must be followed which promise to change the paradigm of medicine and will, most likely, take years of intense work.

Moreover, HA can also be used as augmentation of a surgical procedure as proposed by Doral et al. combining microfractures and HA in the treatment of OC defects [56]. Other authors used MSCs and HA aiming to improve surgical results of OC lesions [55, 57, 60]. Synergistic anabolic actions of HA and PRP have been demonstrated [70, 71]. Similarly, a biocompatible carrier-forming HA-based microgel (PlnD1-HA) in order to preserve BMP2 activities has also been tested in vitro [72] and in vivo [73]. Collagen-low molecular weight hyaluronic acid semi-interpenetrating network loaded with gelatin microspheres for cell and growth factor delivery for nucleus pulposus regeneration has also been proposed [74].

6.5 Future Perspectives

HA can be used in isolation, taking advantage of its inherent properties. However, the road for the future will include, from one side, optimization of HA itself (better understanding of adequate formulation, dosage, number of treatments according to the pathology, and the patients' profile). But from another much more ambitious perspective, what we have learnt from this biopolysaccharide and its possibilities and clinical results should be used to develop advanced TERM strategies combining growth factors, cells, scaffolds, bioreactors, nanotechnology, etc. aiming for the ultimate full repair of the tissues and control of injuries/diseases. Some initial steps have been given but a long road needs to be traveled with this goal.

6.6 Final Remarks

Hyaluronic acid is a safe alternative for conservative treatment of several orthopedic conditions.

Intra-articular joint injections (with or without image-assisted application) have proven to be a safe procedure. Rare and mostly self-limited adverse reactions have been reported (mainly transitory pain and swelling).

It has shown fair midterm results in symptomatic control of osteoarthritis in different joints and in different grades of the disease. The highest clinical experience reported comes from the knee and ankle joint.

Promising results have been achieved in the treatment of tendinopathies with peri-tendon injections. Some authors advise for possible deleterious effects from intra-tendon injections.

More high-quality studies are required before further clinical conclusions at this point.

In the future, TERM approaches promise to improve current results, mainly by combining several factors with advanced strategies, specifically designed for each clinical condition.

References

1. Xing D, Wang B, Liu Q, Ke Y, Xu Y, Li Z, Lin J (2016) Intra-articular hyaluronic acid in treating knee osteoarthritis: a PRISMA-compliant systematic review of overlapping meta-analysis. Sci Rep 6:32790. https://doi.org/10.1038/srep32790
2. Puig-Junoy J, Ruiz Zamora A (2015) Socio-economic costs of osteoarthritis: a systematic review of cost-of-illness studies. Semin Arthritis Rheum 44(5):531–541. https://doi.org/10.1016/j.semarthrit.2014.10.012
3. Hunter DJ (2009) Risk stratification for knee osteoarthritis progression: a narrative review. Osteoarthr Cartil/OARS Osteoarthr Res Soc 17(11):1402–1407. https://doi.org/10.1016/j.joca.2009.04.014
4. van Dijk CN, Reilingh ML, Zengerink M, van Bergen CJ (2010) Osteochondral defects in the ankle: why painful? Knee Surg Sports Traumatol Arthrosc Off J ESSKA 18(5):570–580. https://doi.org/10.1007/s00167-010-1064-x
5. Temenoff JS, Mikos AG (2000) Review: tissue engineering for regeneration of articular cartilage. Biomaterials 21(5):431–440
6. Hunziker EB (2000) Articular cartilage repair: problems and perspectives. Biorheology 37(1-2):163–164
7. Pacifici M, Koyama E, Iwamoto M, Gentili C (2000) Development of articular cartilage: what do we know about it and how may it occur? Connect Tissue Res 41(3):175–184
8. Kuettner KE, Cole AA (2005) Cartilage degeneration in different human joints. Osteoarthr Cartil/OARS Osteoarthr Res Soc 13(2):93–103. https://doi.org/10.1016/j.joca.2004.11.006
9. Gelber AC, Hochberg MC, Mead LA, Wang NY, Wigley FM, Klag MJ (2000) Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. Ann Intern Med 133(5):321–328
10. de Vos RJ, van PLJ V, Moen MH, Weir A, Tol JL, Maffulli N (2010) Autologous growth factor injections in chronic tendinopathy: a systematic review. Br Med Bull 95(1):63–77. https://doi.org/10.1093/bmb/ldq006
11. van Sterkenburg MN, van Dijk CN (2011) Injection treatment for chronic midportion Achilles tendinopathy: do we need that many alternatives? Knee Surg Sports Traumatol Arthrosc Off J ESSKA 19(4):513–515. https://doi.org/10.1007/s00167-011-1415-2
12. Snedeker JG, Foolen J (2017) Tendon injury and repair - a perspective on the basic mechanisms of tendon disease and future clinical therapy. Acta Biomater 63:18–36. https://doi.org/10.1016/j.actbio.2017.08.032
13. Weinraub GM (2005) Orthobiologics: a survey of materials and techniques. Clin Podiatr Med Surg 22(4):509–519., v. https://doi.org/10.1016/j.cpm.2005.08.003

14. Correia SI, Pereira H, Silva-Correia J, Van Dijk CN, Espregueira-Mendes J, Oliveira JM, Reis RL (2014) Current concepts: tissue engineering and regenerative medicine applications in the ankle joint. J R Soc Interface R Soc 11(92):20130784. https://doi.org/10.1098/rsif.2013.0784
15. de Mos M, van der Windt AE, Jahr H, van Schie HT, Weinans H, Verhaar JA, van Osch GJ (2008) Can platelet-rich plasma enhance tendon repair? A cell culture study. Am J Sports Med 36(6):1171–1178. https://doi.org/10.1177/0363546508314430
16. DeChellis DM, Cortazzo MH (2011) Regenerative medicine in the field of pain medicine: prolotherapy, platelet-rich plasma therapy, and stem cell therapy—theory and evidence. Tech Reg Anesth Pain Manag 15:74–80
17. Evans CH (2013) Platelet-rich plasma a la carte: commentary on an article by Satoshi Terada, MD, et al.: "use of an antifibrotic agent improves the effect of platelet-rich plasma on muscle healing after injury". J Bone Joint Surg Am 95(11):e801–e802. https://doi.org/10.2106/JBJS.M.00485
18. Luyten FP, Vanlauwe J (2012) Tissue engineering approaches for osteoarthritis. Bone 51(2):289–296. https://doi.org/10.1016/j.bone.2011.10.007
19. Martel-Pelletier J, Wildi LM, Pelletier JP (2012) Future therapeutics for osteoarthritis. Bone 51(2):297–311. https://doi.org/10.1016/j.bone.2011.10.008
20. Qi Y, Feng G, Yan W (2012) Mesenchymal stem cell-based treatment for cartilage defects in osteoarthritis. Mol Biol Rep 39(5):5683–5689. https://doi.org/10.1007/s11033-011-1376-z
21. Nordsletten L (2006) Recent developments in the use of bone morphogenetic protein in orthopaedic trauma surgery. Curr Med Res Opin 22(s1):S13–S17. https://doi.org/10.1185/030079906X80585
22. Myers KR (2013) Trends in biological joint resurfacing. Bone Joint Res 2(9):193–199. https://doi.org/10.1302/2046-3758.29.2000189
23. Pereira H, Ripoll L, Oliveira JM, Reis RL, Espregueira-Mendes J, van Dijk C (2016) A Engenharia de tecidos nas lesões do Desporto. Traumatologia Desportiva. LIDEL, Lisboa
24. Di Giacomo G, De Gasperis N (2015) The role of hyaluronic acid in patients affected by glenohumeral osteoarthritis. J Biol Regul Homeost Agents 29(4):945–951
25. Flores C, Balius R, Alvarez G, Buil MA, Varela L, Cano C, Casariego J (2017) Efficacy and tolerability of Peritendinous hyaluronic acid in patients with supraspinatus Tendinopathy: a multicenter, randomized, controlled trial. Sports Med Open 3(1):22. https://doi.org/10.1186/s40798-017-0089-9
26. Gigante A, Callegari L (2011) The role of intra-articular hyaluronan (Sinovial) in the treatment of osteoarthritis. Rheumatol Int 31(4):427–444. https://doi.org/10.1007/s00296-010-1660-6
27. Van Den Bekerom MP, Mylle G, Rys B, Mulier M (2006) Viscosupplementation in symptomatic severe hip osteoarthritis: a review of the literature and report on 60 patients. Acta Orthop Belg 72(5):560–568
28. Zengerink M, Struijs PA, Tol JL, van Dijk CN (2010) Treatment of osteochondral lesions of the talus: a systematic review. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 18(2):238–246. https://doi.org/10.1007/s00167-009-0942-6
29. Necas J, Bartosikova L, Brauner P, Kolar J (2008) Hyaluronic acid (hyaluronan): a review. Veterinarni Medicina 53(8):397–411
30. Collins MN, Birkinshaw C (2013) Hyaluronic acid based scaffolds for tissue engineering—a review. Carbohydr Polym 92(2):1262–1279. https://doi.org/10.1016/j.carbpol.2012.10.028
31. Witteveen AG, Hofstad CJ, Kerkhoffs GM (2015) Hyaluronic acid and other conservative treatment options for osteoarthritis of the ankle. Cochrane Database Syst Rev 10:CD010643. https://doi.org/10.1002/14651858.CD010643.pub2
32. Bonnet F, Dunham DG, Hardingham TE (1979) Structure and interactions of cartilage proteoglycan binding region and link protein. Biochem J 228:77–85
33. McArthur BA, Dy CJ, Fabricant PD, Valle AG (2012) Long term safety, efficacy, and patient acceptability of hyaluronic acid injection in patients with painful osteoarthritis of the knee. Patient Prefer Adherence 6:905–910. https://doi.org/10.2147/ppa.s27783

34. Stern R, Asari AA, Sugahara KN (2006) Hyaluronan fragments: an information-rich system. Eur J Cell Biol 85(8):699–715. https://doi.org/10.1016/j.ejcb.2006.05.009
35. Stern R, Kogan G, Jedrzejas MJ, Šoltés L (2007) The many ways to cleave hyaluronan. Biotechnol Adv 25(6):537–557. https://doi.org/10.1016/j.biotechadv.2007.07.001
36. Toole BP (2001) Hyaluronan in morphogenesis. Semin Cell Dev Biol 12(2):79–87. https://doi.org/10.1006/scdb.2000.0244
37. Strauss EJ, Hart JA, Miller MD, Altman RD, Rosen JE (2009) Hyaluronic acid Viscosupplementation and osteoarthritis: current uses and future directions. Am J Sports Med 37(8):1636–1644. https://doi.org/10.1177/0363546508326984
38. Toole BP (2004) Hyaluronan: From extracellular glue to pericellular cue. Nat Rev Cancer 4(7):528–539. https://doi.org/10.1038/nrc1391
39. Bollyky P, Bogdani M, Bollyky J, Hull R, Wight T (2012) The role of Hyaluronan and the extracellular matrix in islet inflammation and immune regulation. Curr Diab Rep 12(5):471–480. https://doi.org/10.1007/s11892-012-0297-0
40. Preston M, Sherman L (2011) Neural stem cell niches: critical roles for the hyaluronan-based matrix in neural stem cell proliferation and differentiation. Front Biosci 3:1165–1179
41. Balazs EA, Denlinger JL (1993) Viscosupplementation: a new concept in the treatment of osteoarthritis. J Rheumatol Suppl 39:3–9
42. Moreland LW (2003) Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: mechanisms of action. Arthritis Res Ther 5(2):54–67
43. Lynen N, De Vroey T, Spiegel I, Van Ongeval F, Hendrickx NJ, Stassijns G (2017) Comparison of Peritendinous Hyaluronan injections versus extracorporeal shock wave therapy in the treatment of painful Achilles' Tendinopathy: a randomized clinical efficacy and safety study. Arch Phys Med Rehabil 98(1):64–71. https://doi.org/10.1016/j.apmr.2016.08.470
44. Araujo JP, Silva L, Andrade R, Pacos M, Moreira H, Migueis N, Pereira R, Sarmento A, Pereira H, Loureiro N, Espregueira-Mendes J (2016) Pain reduction and improvement of function following ultrasound-guided intra-articular injections of triamcinolone hexacetonide and hyaluronic acid in hip osteoarthritis. J Biol Regul Homeost Agents 30(4 Suppl 1): 51–62
45. Bannuru RR, Natov NS, Dasi UR, Schmid CH, McAlindon TE (2011) Therapeutic trajectory following intra-articular hyaluronic acid injection in knee osteoarthritis--meta-analysis. Osteoarthr Cartil/OARS Osteoarthr Res Soc 19(6):611–619. https://doi.org/10.1016/j.joca.2010.09.014
46. Wang CT, Lin J, Chang CJ, Lin YT, Hou SM (2004) Therapeutic effects of hyaluronic acid on osteoarthritis of the knee. A meta-analysis of randomized controlled trials. J Bone Joint Surg Am 86-a(3):538–545
47. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G (2006) Viscosupplementation for the treatment of osteoarthritis of the knee. Cochrane Database Syst Rev (2):Cd005321. https://doi.org/10.1002/14651858.CD005321.pub2
48. Karatosun V, Unver B, Ozden A, Ozay Z, Gunal I (2008) Intra-articular hyaluronic acid compared to exercise therapy in osteoarthritis of the ankle. A prospective randomized trial with long-term follow-up. Clin Exp Rheumatol 26(2):288–294
49. Migliore A, Bizzi E, Massafra U, Vacca F, Alimonti A, Iannessi F, Tormenta S (2009) Viscosupplementation: a suitable option for hip osteoarthritis in young adults. Eur Rev Med Pharmacol Sci 13(6):465–472
50. Monfort J, Rotes-Sala D, Segales N, Montanes FJ, Orellana C, Llorente-Onaindia J, Mojal S, Padro I, Benito P (2015) Comparative efficacy of intra-articular hyaluronic acid and corticoid injections in osteoarthritis of the first carpometacarpal joint: results of a 6-month single-masked randomized study. Joint Bone Spine 82(2):116–121. https://doi.org/10.1016/j.jbspin.2014.08.008
51. Porcellini G, Merolla G, Giordan N, Paladini P, Burini A, Cesari E, Castagna A (2015) Intra-articular glenohumeral injections of HYADD(R)4-G for the treatment of painful shoulder osteoarthritis: a prospective multicenter, open-label trial. Joints 3(3):116–121. https://doi.org/10.11138/jts/2015.3.3.116

52. Legre-Boyer V (2015) Viscosupplementation: techniques, indications, results. Orthop Trauma Surg Res OTSR 101(1s):S101–s108. https://doi.org/10.1016/j.otsr.2014.07.027
53. Braithwaite GJ, Daley MJ, Toledo-Velasquez D (2016) Rheological and molecular weight comparisons of approved hyaluronic acid products - preliminary standards for establishing class III medical device equivalence. J Biomater Sci Polym Ed 27(3):235–246. https://doi.org/10.1080/09205063.2015.1119035
54. Ayhan E, Kesmezacar H, Akgun I (2014) Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. World J Orthop 5(3):351–361. https://doi.org/10.5312/wjo.v5.i3.351
55. Buda R, Vannini F, Cavallo M, Baldassarri M, Luciani D, Mazzotti A, Pungetti C, Olivieri A, Giannini S (2013) One-step arthroscopic technique for the treatment of osteochondral lesions of the knee with bone-marrow-derived cells: three years results. Musculoskelet Surg 97(2):145–151. https://doi.org/10.1007/s12306-013-0242-7
56. Doral MN, Bilge O, Batmaz G, Donmez G, Turhan E, Demirel M, Atay OA, Uzumcugil A, Atesok K, Kaya D (2012) Treatment of osteochondral lesions of the talus with microfracture technique and postoperative hyaluronan injection. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 20(7):1398–1403. https://doi.org/10.1007/s00167-011-1856-7
57. Giannini S, Buda R, Battaglia M, Cavallo M, Ruffilli A, Ramponi L, Pagliazzi G, Vannini F (2013) One-step repair in talar osteochondral lesions: 4-year clinical results and t2-mapping capability in outcome prediction. Am J Sports Med 41(3):511–518. https://doi.org/10.1177/0363546512467622
58. Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, Fornasari PM, Giannini S, Marcacci M (2011) Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. Arthroscopy J Arthrosc Relat Surg Off Publ Arthrosc Assoc North Am Int Arthrosc Assoc 27(11):1490–1501. https://doi.org/10.1016/j.arthro.2011.05.011
59. Mason LW, Wilson-Jones N, Williams P (2014) The use of a cell-free chondroinductive implant in a child with massive cartilage loss of the talus after an open fracture dislocation of the ankle: a case report. J Pediatr Orthop 34(8):e58–e62. https://doi.org/10.1097/bpo.0000000000000198
60. Wong KL, Lee KB, Tai BC, Law P, Lee EH, Hui JH (2013) Injectable cultured bone marrow-derived mesenchymal stem cells in varus knees with cartilage defects undergoing high tibial osteotomy: a prospective, randomized controlled clinical trial with 2 years' follow-up. Arthroscopy J Arthrosc Relat Surg Off Publ Arthrosc Assoc North Am Int Arthrosc Assoc 29(12):2020–2028. https://doi.org/10.1016/j.arthro.2013.09.074
61. Colen S, Geervliet P, Haverkamp D, Van Den Bekerom MP (2014) Intra-articular infiltration therapy for patients with glenohumeral osteoarthritis: a systematic review of the literature. Int J Shoulder Surg 8(4):114–121. https://doi.org/10.4103/0973-6042.145252
62. Trellu S, Dadoun S, Berenbaum F, Fautrel B, Gossec L (2015) Intra-articular injections in thumb osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. Joint Bone Spine 82(5):315–319. https://doi.org/10.1016/j.jbspin.2015.02.002
63. Abat F, Alfredson H, Cucchiarini M, Madry H, Marmotti A, Mouton C, Oliveira JM, Pereira H, Peretti GM, Romero-Rodriguez D, Spang C, Stephen J, van Bergen CJA, de Girolamo L (2017) Current trends in tendinopathy: consensus of the ESSKA basic science committee. Part I: biology, biomechanics, anatomy and an exercise-based approach. J Exp Orthop 4(1):18. https://doi.org/10.1186/s40634-017-0092-6
64. Frizziero A, Vittadini F, Barazzuol M, Gasparre G, Finotti P, Meneghini A, Maffulli N, Masiero S (2017) Extracorporeal shockwaves therapy versus hyaluronic acid injection for the treatment of painful non-calcific rotator cuff tendinopathies: preliminary results. J Sports Med Phys Fitness 57(9):1162–1168. https://doi.org/10.23736/S0022-4707.16.06408-2
65. Kumai T, Muneta T, Tsuchiya A, Shiraishi M, Ishizaki Y, Sugimoto K, Samoto N, Isomoto S, Tanaka Y, Takakura Y (2014) The short-term effect after a single injection of high-molecular-weight hyaluronic acid in patients with enthesopathies (lateral epicondylitis, patellar tendi-

nopathy, insertional Achilles tendinopathy, and plantar fasciitis): a preliminary study. J Orthop Sci 19(4):603–611. https://doi.org/10.1007/s00776-014-0579-2
66. Wu PT, Jou IM, Kuo LC, Su FC (2016) Intratendinous injection of hyaluronate induces acute inflammation: a possible detrimental effect. PLoS One 11(5):e0155424. https://doi.org/10.1371/journal.pone.0155424
67. Antunes JC, Oliveira JM, Reis RL, Soria JM, Gomez-Ribelles JL, Mano JF (2010) Novel poly(L-lactic acid)/hyaluronic acid macroporous hybrid scaffolds: characterization and assessment of cytotoxicity. J Biomed Mater Res A 94(3):856–869. https://doi.org/10.1002/jbm.a.32753
68. Forriol F, Longo UG, Duart J, Ripalda P, Vaquero J, Loppini M, Romeo G, Campi S, Khan WS, Muda AO, Denaro V (2015) VEGF, BMP-7, Matrigel(TM), hyaluronic acid, in vitro cultured chondrocytes and trephination for healing of the avascular portion of the meniscus. An experimental study in sheep. Curr Stem Cell Res Ther 10(1):69–76
69. Kon E, Filardo G, Robinson D, Eisman JA, Levy A, Zaslav K, Shani J, Altschuler N (2013) Osteochondral regeneration using a novel aragonite-hyaluronate bi-phasic scaffold in a goat model. Knee Surg Sports Traumatol Arthrosc. [Epub ahead of print]:1–13. https://doi.org/10.1007/s00167-013-2467-2
70. Anitua E, Sanchez M, De la Fuente M, Zalduendo MM, Orive G (2012) Plasma rich in growth factors (PRGF-Endoret) stimulates tendon and synovial fibroblasts migration and improves the biological properties of hyaluronic acid. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 20(9):1657–1665. https://doi.org/10.1007/s00167-011-1697-4
71. Chen WH, Lo WC, Hsu WC, Wei HJ, Liu HY, Lee CH, Tina Chen SY, Shieh YH, Williams DF, Deng WP (2014) Synergistic anabolic actions of hyaluronic acid and platelet-rich plasma on cartilage regeneration in osteoarthritis therapy. Biomaterials 35(36):9599–9607. https://doi.org/10.1016/j.biomaterials.2014.07.058
72. Srinivasan PP, McCoy SY, Jha AK, Yang W, Jia X, Farach-Carson MC, Kirn-Safran CB (2012) Injectable perlecan domain 1-hyaluronan microgels potentiate the cartilage repair effect of BMP2 in a murine model of early osteoarthritis. Biomed Mater 7(2):024109. https://doi.org/10.1088/1748-6041/7/2/024109
73. Sanchez M, Azofra J, Anitua E, Andia I, Padilla S, Santisteban J, Mujika I (2003) Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. Med Sci Sports Exerc 35(10):1648–1652. https://doi.org/10.1249/01.MSS.0000089344.44434.50
74. Tsaryk R, Gloria A, Russo T, Anspach L, De Santis R, Ghanaati S, Unger RE, Ambrosio L, Kirkpatrick CJ (2015) Collagen-low molecular weight hyaluronic acid semi-interpenetrating network loaded with gelatin microspheres for cell and growth factor delivery for nucleus pulposus regeneration. Acta Biomater 20:10–21. https://doi.org/10.1016/j.actbio.2015.03.041