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Regenerative approaches for the treatment of early OA

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Abstract The diagnosis and the prompt treatment of early osteoarthritis (OA) represent vital steps for delaying the onset and progression of fully blown OA, which is the most common form of arthritis, involving more than 10 % of the world's population older than 60 years of age. Nonsurgical treatments such as physiotherapy, anti-inflammatory medications, and other disease-modifying drugs all have modest and short-lasting effect. In this context, the biological approaches have recently gained more and more attention. Growth factors, blood derivatives, such as platelet concentrates, and mesenchymal adult stem cells, either expanded or freshly isolated, are advocated amongst the most promising tool for the treatment of OA, especially in the early phases. Primarily targeted towards focal cartilage defects, these biological agents have indeed recently showed promising results to relieve pain and reduce inflammation in patients with more advanced OA as well, with the final aim to halt the progression of the disease and the need for joint replacement. However, despite of a number of satisfactory in vitro and pre-clinical studies, the evidences are still limited to support their clinical efficacy in OA setting.
Level of evidence V.

Keywords Osteoarthritis · Molecular therapy · Growth factors · Cartilage · Mesenchymal stem cells

Introduction

Osteoarthritis (OA) is the most common of the arthropathies, with a prevalence of clinically defined OA estimated to be approximately 10 % of the world's population aged 60 years or older, thus representing one of the major sources of pain, disability, and socioeconomic cost worldwide [19, 57]. OA may be initiated by various mechanisms of onset or conditions, and, ultimately, result in a common end point. One of the most common stratifications of OA is mechanism of onset, consisting of primary (idiopathic)

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versus secondary OA, the latter mainly induced by trauma and thus referred to as post-traumatic OA [13]. Independently form its aetiology, the timely diagnosis of early OA [35] is crucial for the prompt setup of the adequate therapy, which has the purpose of delaying or sometimes interrupting its evolution. If not properly recognized and treated in its first phases, in fact, it may evolve towards symptomatic and advanced OA. However, one of the main reasons for the increase in demand for the consequent knee and hip replacement is that conservative treatments for OA are not very effective. Indeed, nonsurgical treatments such as physiotherapy, anti-inflammatory, and anti-pain medications all have modest and short-lasting efficacy at best. In particular, early onset of OA poses special therapeutic challenges for young patients; thus, the reward for any prevention strategy or delay of the disease is very relevant. In this context, the biological approaches have recently gained more and more attention due to their anti-inflammatory and immunomodulatory properties, regenerative potential, and high tolerability. Specifically, adult stem cells of different origins, due to their ability to act not only on cartilage but on the whole joint, are advocated as the best potential tool for the treatment of OA [8]. However, despite promising findings in both in vitro and pre-clinical biological studies, there is still a lack of clear evidences to support clinical efficacy of growth factors, platelet concentrates, and pluripotent stem cells in OA.

Growth factors

Growth factors (GFs), either produced by the cell or administered externally, play a significant role in musculoskeletal tissues homeostasis and repair. One of the most developed directions in biological treatment of post-traumatic OA (PTOA) is the use of GFs due to their ability to stimulate cartilage matrix synthesis and pro-anabolic responses in chondrocytes. Some of them have been studied for more than three decades [65]. Amongst them are the members of the transforming growth factor-β (TGF-β) superfamily, especially bone morphogenetic proteins (BMPs), fibroblast growth factors (FGF)-2 and 18, and insulin-like growth factor-1 (IGF-1) [45]. BMP-2 and BMP-7 appear to be extremely potent in cartilage and bone repair [9, 23]. They are expressed in the majority of musculoskeletal tissues and can be regulated genetically or epigenetically; they crosstalk with a variety of signalling pathways and regulate multiple catabolic and anabolic processes [8]. Their effects depend on endogenous levels, doses, and formulations of recombinant proteins, cell type used for cartilage repair or delivery of GF, scaffold, delivery mode, treatment regimen, intraarticular environment, joint biomechanical integrity, and other [65]. Their regenerative potential has been documented in numerous small and large animal models of post-traumatic OA or degenerative osteoarthritis OA [53]; yet, there are very few completed human clinical trials with satisfactory results [33]. Tissue Gen. Inc., has recently developed TG-C (cartilage), which consists of allogeneic chondrocytes that have been genetically modified to produce the therapeutic growth factor (TGF-β1). At the moment, there is a Phase II study in the USA being conducted for the treatment of knee OA where these modified cells are intraarticularly injected (clinical trials.gov/NCT 01221441), and initial results seem to be promising [53]. Recent developments with blood derivatives and a limited success of pre-clinical and clinical trials with various GF indicate a potential paradigm shift in GF therapy for cartilage repair. It might be more reasonable to consider a cocktail of GFs rather than a single GF treatment applied at different phases of cartilage repair process (Fig. 1). Important to consider are also the time of treatment after injury or onset of symptoms, availability of cells with regenerative potential, pathology and appropriateness of GF therapy, patient's health history, and other parameters. The goal should be creating a healing environment, in which appropriate cells provide sustained release of multiple GF at the required stages of the healing process.

Blood derivatives

Blood derivatives have been recently advocated as safe, easy, cost-effective, and minimally invasive strategy to provide bioactive molecules able to influence the joint environment favouring the restoration of a homeostatic balance and possibly the regeneration of degenerating tissues.

The first attempt to generate a blood-derived product for intraarticular use was developed in the mid-1990s: autologous conditioned serum (ACS) was proposed as injectable material enriched with endogenous IL-1 receptor antagonist (IL-1Ra) to limit the effect of IL-1, the most potent known catabolic mediator of cartilage loss in osteoarthritic joints. The use of ACS has been investigated in a double-blind randomized controlled trial (RCT) on patients with knee OA, showing that ACS injections considerably improved the clinical signs and symptoms of OA with results even superior to those of HA [2]. The study focused on the treatment of patients with Kellgren–Lawrence grades 2–3 OA, a more advanced stage of OA; however, since IL-1Ra might have a protective role in the entire OA process, these results suggest the usefulness of ACS injections for the treatment of early OA too. Yet, despite encouraging clinical findings, concerns have been raised on the real usefulness of this blood derivative, with both evidence of limited intraarticular

effect on cytokine level and simultaneous presence of anti- and pro-inflammatory mediators. There was also a lack of in vitro effect on cartilage metabolism [58].

More recently, another blood-derived product, platelet-rich plasma (PRP), has gained increasing attention. Due to the GFs stored in platelet α-granules, found to regulate articular cartilage metabolism [15], platelet concentrates have been proposed as a simple and minimally invasive method for injection of a high concentrate of autologous GFs and other bioactive molecules in physiological proportions [32]. Beside an extensive literature with positive reports on PRP use, only a few high-level studies have been currently published. Existing RCTs present an overall support to PRP injections for knee OA treatment showing an early beneficial effect slightly superior to what was obtained with viscosupplementation [15]. They also provide the evidence of superiority in comparison with placebo [54]. Importantly, outcomes depend on age and the level of cartilage degeneration with better results in younger patients with early OA. Unfortunately, limited duration of the beneficial effect, roughly 6–12 months, does not support a regenerative effect on articular cartilage, but rather suggests a temporary homeostatic improvement of the joint environment, especially in less advanced cases [15]. Lately, the potential of PRP and the superiority with respect to hyaluronic acid have been questioned by a double-blind RCT on a large cohort of patients, which documented a similar response to treatments in 12-month follow-up including subanalysis of patients with early phase of knee degeneration [14].

While PRP still appears to be an attractive choice for early OA due to an overall positive biological and clinical findings, more robust studies are needed to support the efficacy of PRP injections by identifying the most suitable preparation procedure of the platelet concentrate [5], the best application modality, and the most responsive patients and disease phases. In fact, this research area is still in its infancy, and a huge gap yet to be filled in order to understand how to translate the biological rational of PRP into a proven clinical benefit.

Mesenchymal stem cells

Source

Over the last decade, MSCs have become an attractive alternative for the treatment of OA due to their trophic, immunomodulatory, and anti-inflammatory effect exhibited through direct cell–cell interaction or secretion of bioactive molecules [46]. Indeed, either as direct partners of repair process or/and growth factors "drug stores", MSCs may enhance tissue repair and regeneration.

MSCs are ubiquitous in the body, deriving from perivascular cells called pericytes, but specific sites have been identified as particularly favourable in order to obtain a considerable number of cells to be used for bone and cartilage repair. However, differentiation potential is dependent on several factors such as architectural extracellular and intercellular segmental characterization, environmental factors, growth factors, and adequate pool of MSCs [40]. The "oldiest goldiest" is represented by the bone marrow (BM). Bone marrow-derived stem cells (BMSCs) have demonstrated to be effective in the treatment of large cartilage lesions in the knee in the form of BM concentrate (non-expanded cells) [20], as well as in form of expanded BMSC in the treatment of knee OA [63]. The subcutaneous adipose tissue has recently gained attention as a source of MSCs due to a simple and less-invasive method of harvesting. Some concerns have been raised about a putative inferior chondrogenic potential of these cells named ASCs (adipose-derived stem cells) [24]. However, as reported by Jo et al. [25], reasonable cartilage regeneration has been achieved with the use of arthroscopic injections of culture-expanded ASCs in OA knee or one-step administration of stromal vascular fraction (SVF) (i.e. fraction of adipose tissue containing different cell types including ASCs) for the treatment of focal large cartilage lesions [29]. Infrapatellar Hoffa fat pad has been also advocated as a smart source of MSCs, both for its accessibility during knee surgery and the higher chondrogenic potential of these cells (IFP-MSCs) if compared to ASCs [34]. Amongst the newest candidates, peripheral blood (PB) appears to have a promising position. When stimulated by cytokines as G-CSF (granulocytes colony stimulating factor), peripheral blood hosts a considerable number of precursor cells [39]. These cells may contribute to cartilage repair, as demonstrated by a recent clinical trial showing the treatment of full-thickness chondral lesion by arthroscopic subchondral drilling and post-operative intraarticular injections of autologous PB-MSCs in combination with HA [60]. In this regard, the role of G-CSF both as a trophic factor and as an efficient device for systemic mobilization of precursor cells has to be considered. Albeit some obvious difficulties with harvesting technique, expanded synovial tissue-derived MSCs have also been proposed as potential candidates for knee chondral repair and showed promising results when directly injected after arthroscopic debridement at the lesion site [61]. Muscle-derived stem cells (MDSCs) have been also recently recognized as a potential tool for cartilage repair. In an OA-like model in nude rats, the use of MDSC transduced with retroviral vectors encoding BMP-4 and sFlt1 (a vascular endothelial growth factor-VEGF antagonist) combined with PRP produced encouraging results [44]. Lastly, the umbilical cord (UC) has been included as a potential source of mesenchymal stem cells, both from the cord blood and the stroma [41]. A recent study demonstrated the feasibility and the safety of the use of allogeneic

UC-derived mesenchymal stem cells for the treatment of focal chondral defects at 72-month follow-up [4]. Although MSCs can be isolated from several tissues, not all MSCs offer the same therapeutic potential; for example, the in vitro chondrogenic potential of BMSCs has been found to be higher than those of ASCs [1].

Expanded or concentrated MSCs

The potential of MSCs can be exploited by using either expanded cells or concentrated progenitor pools. The use of expanded stem cells allows a more reproducible treatment, as it is possible to isolate a purer MSC population expressing CD105, CD73, and CD90, and lacking expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR on their surface. Moreover, it permits a better assessment of the exact number of cells used in each treatment, thus assuring a better control of this approach (Fig. 2). However, the treatment with autologous expanded cells is a two-step procedure; it is more invasive and of a higher cost. Furthermore, the need for extensive cell manipulation transforms expanded cells into an advanced-therapy medicinal product (ATMPs) subjected to more rigorous regulatory requirements for their use in clinical practice. As a result, a number of different devices for the intraoperative concentration of both BM and adipose tissue-derived progenitor cells have been developed and are commercially available today. These progenitor cell concentrates are easier to use; they have all advantages of a one-step surgery procedure, though containing a lower number of MSCs in comparison with expanded cell suspension and differing markedly in composition.

From the biological point of view, the main difference between the use of expanded MSCs and progenitor cell concentrates is the homogeneity of cell population. Cell passaging allows obtaining a very homogeneous population of cells composed primarily of MSCs, >95 %; whereas, progenitor cell concentrates contain different cell types, so-called BM or adipose tissue niche. Indeed, the intraoperative methods permit obtaining the BM aspirate concentrate (BMAC) composed of lymphocytes granulocytes, blast cells and erythrocytes for 90 %, and by monocytes for 10 % (of which only about 1 % are MSCs) [11]. However, many studies demonstrate that mesenchymal and haematopoietic stem cells form a unique BM niche and that their co-presence is required to obtain the best tissue regeneration results [42]. Similarly, the progenitor cell-rich product obtained intraoperatively from adipose tissue, named SVF, contains preadipocytes, vascular endothelial cells, smooth muscle cells and pericytes (ASCs), leucocytes, and erythrocytes. In this case, the maintenance of the stromal cell niche architecture seems to be a great advantage [17].

Despite these observations, both expanded MSCs and MSC-rich concentrates from BM and adipose tissues have shown promising clinical results in the treatment of cartilage defects including OA.

Autologous or allogeneic MSCs

MSCs are non-immunogenic due to their low expression of antigen-presenting molecules [56]. They are also characterized by immunomodulatory/immunosuppressive properties, making them useful in the treatment of steroid-resistant graft-versus-host disease, acute respiratory distress syndrome, and Crohn's disease [47]. Despite these properties, several concerns have made the clinical use of allogeneic MSCs very limited to date, including the possibility that once differentiated, MSCs lose their immunogenicity and immunomodulatory properties [59]. Many pre-clinical studies in different animal models demonstrated the feasibility and the safety of the use of allogeneic cells in the treatment of cartilage injuries [3, 12]. Indeed, due to the lack of vascular lymphatic systems cartilage is particularly immunoprivileged. However, when debridement or microfractures are used to treat cartilage defects, the influx of BM might become a concern for using allogeneic cells. Despite the high number of studies using allogeneic MSCs in different clinical applications, the first clinical studies exploring the application of allogeneic MSCs for cartilage repair started only recently. A phase II clinical trial using umbilical mesenchymal stem cells combined with sodium hyaluronate for the treatment of articular cartilage defects was carried out in Korea (CARTISTEM®, MEDIPOST, Korea). The efficacy and safety of this approach has been demonstrated in a 72-month follow-up study that also showed the repair tissue resembling hyaline-like cartilage [4]. The same product is currently under investigation in a phase I/II clinical trial in the USA for the treatment of ICRS grade 3 or 4 focal cartilage defects larger than 2 cm^2 (clinical trials.gov/NCT01733186). A different idea was the base of an ongoing phase I/II clinical trial in the Netherlands, in which rapidly isolated autologous chondrocytes together with their pericellular matrix (chondrons) were combined with allogeneic BMSCs in fibrin glue to treat focal articular cartilage defects [3]. Initial data produced no immunological concerns, thus supporting the safety of allogeneic approach (clinical trials.gov/NCT02037204). To date, only two clinical studies using allogeneic BMSCs for the treatment of OA have been published [38, 63]. Both groups showed that BMSCs were effective in pain reduction which hopefully can also lead to structural improvements resulting in the arrest of the disease progression and enhanced cartilage regeneration. Nonetheless, ex vivo-cultured allogeneic MSCs are currently being tested for efficacy and safety, which may broaden their clinical utility.

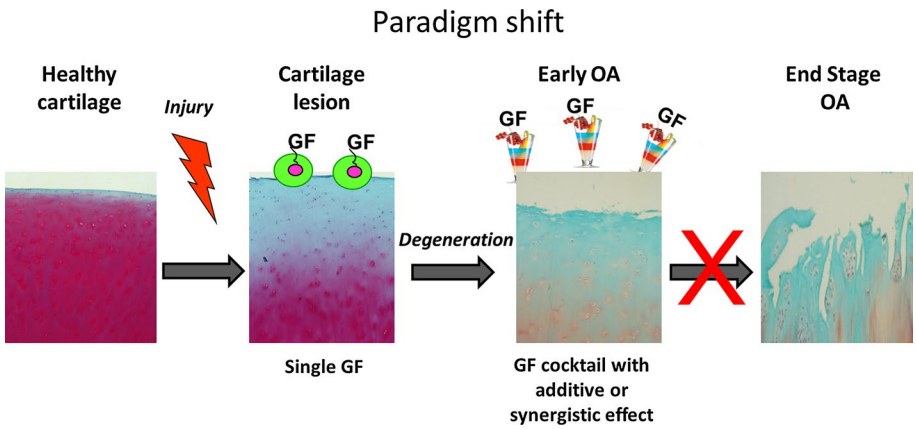

Fig. 1 Treatment paradigm shift: from a single growth factor to a cocktail of growth factors

Injective treatment with cell concentrates

Overview

Intraarticular injections of cell concentrates for the treatment of early OA offer great advantages by allowing cells within joint space to target the injured tissues through interaction with recipient cells and surfaces eventually leading to better outcomes [46]. In addition, such approach is minimally invasive, is cost-effective, and has a better patient compliance (Fig. 3).

The use of expanded MSCs introduces concerns related to manipulation. In different countries, they are considered drugs, which complicates their clinical utilization due to severe regulatory requirements. Moreover, the danger of bacterial contamination, xenogenic risk, or cellular transformation, also influencing the differentiation capacities of MSCs, represents additional hurdles. One of the major concerns is the application protocol that includes the most effective cell dosage, the number and timing of injections. The dose–response relationship of MSCs transplantation for clinical cartilage repair has not yet been established. The current literature differs in cell quantities and the number of injections making comparison of clinical outcomes very difficult [16].

There are also attempts to improve the effect of MSCs. Several strategies are currently used in clinical practice, such as the combination with PRP or HA [27, 29]. The results are encouraging, although it is difficult to define the real effects of these substances due to several limitations in study design. Other kinds of augmentation with therapeutic agents (growth, transcription, or signalling factors), provided as peptides or genetic sequences, are currently under active investigation in the preclinical setting [16]. Lastly, little is known about the patient's profile that may benefit the most from this kind of treatment. Certainly, patient age and degree of joint degeneration play an important role [27]. However, the lack of commonly accepted guidelines justifies the need for high-quality trials necessary to address these and other concerns.

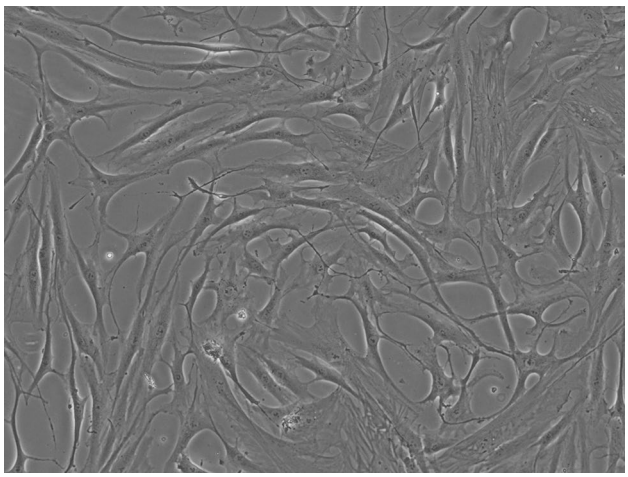

Fig. 2 ASCs (adipose-derived stem cells) cultivated for 15 days in non-inductive culture medium

Bone marrow

Injection of expanded BMSC may constitute a possible alternative in treatment of knee OA, as shown by recent clinical trials [63]. The encouraging results obtained in animal models demonstrating the safety and feasibility of the use of expanded BMSCs [55] allowed translating this approach to patients. In a pilot knee OA study (EudraCT 2009-017407-11 and clinical trials.gov/NCT01183728), 12 patients were treated by a single intraarticular injection of 40 x 10^6 autologous expanded BMSC. Pain functional scores and radiological findings suggested good outcomes with the absence of adverse side effects [51]. The same group has recently published the results of a 12-month follow-up on the first 50 patients who underwent the same procedure as in the pilot study [62]. In all patients, the

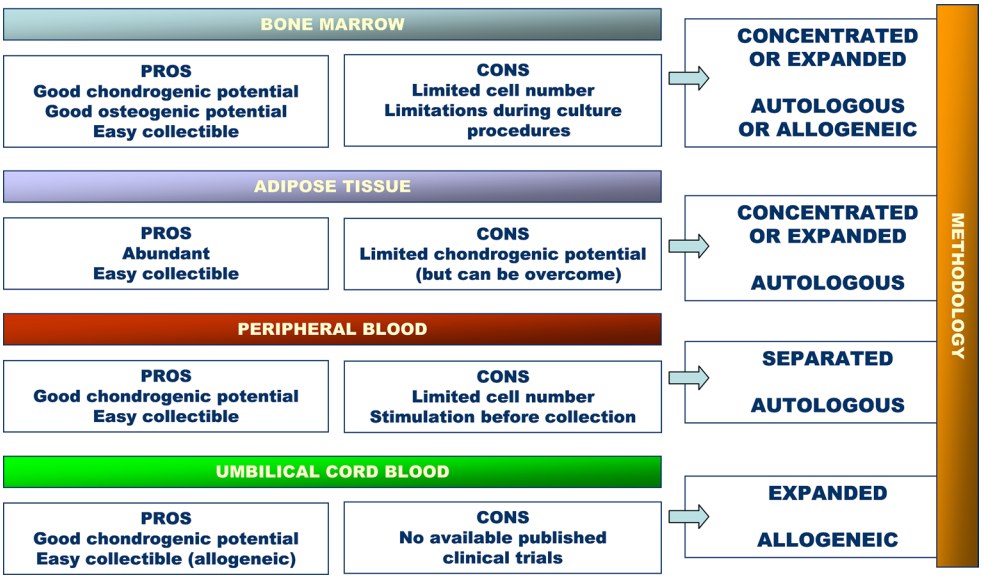

Fig. 3 MSCs in clinical practice for injective treatment, an overview

treatment produced satisfactory results in terms of pain reduction and functional recovery during daily activities including recreational sports. In T2 mapping MRI evaluation, the Poor Cartilage Index (PCI), calculated to assess cartilage quality, showed a significant decrease in all patients. Kim et al. treated 41 patients (75 knees) with different grades of knee OA (92 % of patients had Kellgren–Lawrence grades I–III) with a single autologous BMAC injection (10 ml of lipoaspirate). After 12 months, pain and functional scores were significantly improved in comparison with the pre-injection level, particularly in those patients affected by early to moderate OA [26]. These findings were supported by another study with a larger number of patients: 618 single autologous injections of BMAC and PRP were performed in patients affected by knee OA (80 % Kellgren–Lawrence grades I–II). At 12-month follow-up, significant clinical improvements were observed in regards to pain and functional scores [6]. In the same study, further 214 patients were treated with the same procedure in association to lipoaspirate, but with no additional benefits deriving from this association.

Due to inconsistent findings, additional studies are necessary to evaluate different doses of cells and carriers to enhance cell viability and efficacy. Nevertheless, at this stage of our knowledge, the injective treatment with autologous expanded BMSCs and BMAC can be considered a feasible, safe, and effective treatment for early OA.

Adipose tissue

Although ASCs have been demonstrated to exert an anti-inflammatory and chondroprotective effects in many models of experimental OA, few clinical studies have been published thus far.

In a phase I/II study, Jo et al. showed that intraarticular injections of autologous expanded ASCs in osteoarthritic knees of 18 patients enhanced better function, reduced pain, and lessened cartilage defects by regeneration of hyaline-like articular cartilage at 6-month follow-up without causing adverse events. Moreover, a dose-dependent effect has been demonstrated with the best outcome at the highest amount of ASCs (1×10^8) [25].

In a comparative study, the effect of a single injection after debridement of PRP or PRP + SVF cells derived from infrapatellar fat pad was evaluated in patients with knee OA. The results showed no significant differences between the two groups, though the pre-operative scores of patients belonging to PRP + SVF cells group were significantly poorer than those of the patients of control group [28].

Koh et al. [30] showed the effectiveness of SVF cells (mean 1.18×10^6 cells) derived intraoperatively from infrapatellar fat pad of 18 patients at 2-year follow-up. All clinical parameters evaluated were significantly improved. Moreover, at the final follow-up, the whole-organ MRI score (WORMS) was significantly better than the baseline value. Importantly, identified improvements in clinical and MRI results positively correlated with the number of injected ASCs.

More recently, the same authors published the results of a similar study on 30 patients with Kellgren–Lawrence grades II–III knee OA. Patients were administered 4×10^6 expanded ASCs isolated from infrapatellar fat pad [29]. Besides significant improvements in pain relief and functional recovery, this study showed that in

16 patients who had a second-look arthroscopy at 2-year follow-up, 62 % of patients scored "very positive" and "positive". Interestingly, only 16 % of patients had a worsened Kellgren–Lawrence OA grade during the observation period, though none of them underwent total knee arthroplasty during this 2-year period. Pak et al. [52] also showed the effectiveness of one injection of SVF isolated from abdominal area on 91 patients at 30-month follow-up. The superiority of one injection of SVF with respect to PRP alone in case of patients treated with HTO was also reported [31].

In a very large study, the results of 1824 intra/periarticular injections of SVF derived from subcutaneous adipose tissue in patients affected by Kellgren–Lawrence grades II–IV OA, of which 45 % were candidates for arthroplasty, were reported [43]. Patients were administered a single autologous SVF injections and evaluated at a mean follow-up of 17 months. The results showed no serious side effects and 91 % of patients with significant improvements in pain and function. Interestingly, older patients scored better than younger ones; moreover, subtle but significant widening of joint spaces was observed on X-ray in most patients and smoothing of surface irregularities, regression of reactive subchondral bone oedema, sealing of chondral fissures, healing of subchondral cortical lesions, or integration of chondral flaps was also found.

Together, these studies demonstrate safety and feasibility of both expanded ASCs and SVF cells for the treatment of OA, although many issues, including the amount of cells, the number of injections and the ideal patient characteristics, still need to be clarified.

Surgical treatment

While traditionally not indicated for the treatment of OA, surgical cartilage repair has been used for some years to treat focal cartilage lesions due to its potential to control pain and alter the progression of degenerative disease, with the hope of delaying or obviating the onset of a more severe OA [22].

Both expanded MSCs and progenitor cell concentrates have been used in association to different matrices for the surgical treatment of patients affected by cartilage defects. Most of the studies used BMAC, but recently adipose tissues in the form of SVF have been also utilized. Although most of the studies are of low level (IV or V), the literature suggests that MSCs, expanded or not, are a useful tool for cartilage repair [18, 21, 48, 64]. Indeed, all reported clinical improvement with a follow-up period ranging from 1 to 5 years [64] demonstrated a complete defect fill [20, 64], good integration with the surrounding native cartilage [21], and that the reparative tissue was hyaline-like [21, 48], fibrocartilage [64], or a mixture of both [18]. These findings are confirmed by a comparative cohort study, where the efficacy of autologous chondrocyte implantation was compared with implantation of expanded BMSCs at 2 year follow-up [48]. It was concluded that BMSCs were as effective as chondrocytes and, interestingly, patients' age negatively affected the treatment outcome in the ACI group, but not in the BMSC one. Bone marrow aspirate concentrate (BMAC) was demonstrated to be effective in treating large cartilage lesion in the knee at minimum 3-year follow-up in combination with a collagen type I/III matrix or hyaluronan-derived scaffold (HA) [20]. The lack of comparative studies about expanded BMSCs and BMAC confines a prediction to what the best option for MSC-based cartilage repair would be. Moreover, due to the non-homogeneity amongst studies in terms of type of cell carriers, passages, and doses, much remains to be investigated and understood.

ASCs were also demonstrated to be effective in the treatment of cartilage defects, but conclusive clinical results have yet to be published. Nonetheless, studies with the use of ASCs covered by autologous periosteal membrane or acellular collagen dermal matrix for the treatment of cartilage defects are in progress (clinical trials.gov/NCT01399749, NCT02090140).

Future directions

A better knowledge of the natural history of early OA will identify new targets for intervention [36]. Particularly, for treating early OA—where structural damage may be reversible—new molecules such as GFs delivered as proteins will be tested in clinical trials. For example, intraarticular FGF-18 significantly reduced cartilage loss in the lateral tibiofemoral compartment, although no effects were seen in the medial side [33]. Clinical investigations on gene therapy for OA are ongoing, e.g. by intraarticularly injecting TGF-β overexpressing chondrocytes [37]. Future clinical trials should be designed with higher specificity targeting a better-defined patient cohort. Ideally, patients enrolled in such clinical trials should present early OA in the same region of the knee joint, have comparable underlying pathologies that led to OA, and have similar (axial) alignment. Thus, early OA may serve as a perfect model to generate necessary information needed for an improved understanding of processes that control early cartilage degeneration and regeneration, and to

Table 1 Future directions: challenges and solutions

Future directions	Challenges and solutions
Better insight into mechanisms of the disease	Will be the basis for improved therapies. For example, studies of articular fracture shed important light on the role of cytokines at the time of early OA induction and progression. As several pro-inflammatory cytokines and related inflammatory mediators are elevated [50], anti-cytokine, e.g. interleukin 1 receptor antagonist delivery, was shown to reduce the severity of early OA in animal models [49]. Clinical trials will help to define this approach of counteracting the early intraarticular inflammatory response
Identification of new therapeutic molecules	Potential factors include *SOX9*, FGF-2, TGF-β, or IGF-I [10]. FGF-18 has recently shown individual effects for each tibiofemoral knee joint compartment [33]. Specifically, it significantly reduced cartilage loss in the lateral tibiofemoral compartment, while no effects were seen in the medial side. It is well known that the status of cartilage differs between the medial and lateral tibiofemoral compartments, with the medial compartment being more commonly affected by cartilage damages [7]
Gene therapy approaches	Clinical improvements were seen in randomized placebo-controlled trials of knee OA treated with intraarticular injection of human juvenile allogeneic chondrocytes overexpressing a cDNA encoding transforming growth factor-beta-1 via retroviral vectors. No structural cartilage modification demonstrated yet
Clinical trials	Might be designed to be more specific for the region of interest within the knee. For example, not only the tibiofemoral compartment of interest may have to be defined, but also the compartmental subregion (e.g. submeniscal or central) to precisely detect possible treatment effects. Similarly, the variety of individual pathological pathways that may induce (post-traumatic) OA (chondral, subchondral or osteochondral injuries, ligamentous instability, single or repeated direct impact, intraarticular fracture resulting in pre-osteoarthritic deformities, meniscal tears) will have to be specifically addressed. Similar degrees of (axial) alignment needed, as treatment effects (in the medial tibiofemoral compartment), may possibly be eradicated by persistent malalignment

develop disease-modifying OA drugs beneficial for the patient. Research and medical communities should combine their efforts in enhancing the arsenal of regenerative approaches for the treatment of patients with early OA (Table 1).

Conclusion

Although the use of biologics was primarily targeted towards focal cartilage defects, recently, they also showed promising results in pain relief and reduced inflammation in patients with OA. Numerous studies are currently in progress to clarify questions that still remain unanswered regarding the long-term durability of these procedures, the possible modifications that have to be done to achieve better results, and the best-performing biological agents for each given kind of patient and/or grade of disease. To accomplish this task, we still need to a have a better understanding of the biology of cartilage repair and to advocate for broader collaborations between industry, academia, and regulatory agencies. In the meanwhile, carefully conducted randomized prospective studies for each of these innovations should be performed to validate their safety first and then efficacy.

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