Biological Therapies in Regenerative Sports Medicine

Isabel Andia1 · Nicola Maffulli2,3

Abstract Regenerative medicine seeks to harness the potential of cell biology for tissue replacement therapies, which will restore lost tissue functionality. Controlling and enhancing tissue healing is not just a matter of cells, but also of molecules and mechanical forces. We first describe the main biological technologies to boost musculoskeletal healing, including bone marrow and subcutaneous fat-derived regenerative products, as well as platelet-rich plasma and conditioned media. We provide some information describing possible mechanisms of action. We performed a literature search up to January 2016 searching for clinical outcomes following the use of cell therapies for sports conditions, tendons, and joints. The safety and efficacy of cell therapies for tendon conditions was examined in nine studies involving undifferentiated and differentiated (skin fibroblasts, tenocytes) cells. A total of 54 studies investigated the effects of mesenchymal stem-cell (MSC) products for joint conditions including anterior cruciate ligament, meniscus, and chondral lesions as well as osteoarthritis. In 22 studies, cellular products were injected intra-articularly, whereas in 32 studies MSC products were implanted during surgical/arthroscopic procedures. The heterogeneity of clinical conditions, cellular products, and approaches for delivery/implantation make comparability difficult. MSC products appear safe in the short- and midterm, but studies with a long follow-up are scarce. Although the current number of randomized clinical studies is low, stem-cell products may have therapeutic potential. However, these regenerative technologies still need to be optimized.

Key Points

- Biologics, including adult cells, platelet-rich plasma, and conditioned media, are under investigation for regenerative purposes in sports medicine.
- Cell therapies under clinical assessment include dermal fibroblasts, tenocytes, chondrocytes, and various products containing stem cells, mainly bone marrow concentrate, stromal vascular fraction, bone marrow-derived mesenchymal stem cells, and adipose stem cells.
- The use of adult cell therapies is safe. Most articles report improvement in clinical outcomes, but overall the quality of evidence is low, with the absence of adequately powered controlled clinical trials.

1 Introduction

Impairment of biological and mechanical homeostasis is common in orthopedic sports medicine where musculoskeletal tissues are often subjected to excessive stresses...
and multiple injuries. Major injuries in athletes result in a high incidence of chronic problems such as osteoarthritis (OA), for which effective treatments are not yet available [1]. Both acute and chronic sports lesions have promoted a surge of novel biological therapies aiming to improve the quality of life of athletes and active individuals.

Currently, most expectations in regenerative sports medicine are focused on the potential of cell therapies, typically adult mesenchymal stromal/stem cells (MSCs). Regenerative medicine seeks to harness the potential of cell biology for re-establishment of lost tissue functionality. However, controlling and enhancing musculoskeletal tissue regeneration is not just a matter of cells. Molecules, cells, and mechanical forces are hardwired and cooperate in healing mechanisms. For example, platelet-rich plasma (PRP), an autologous regenerative technology, is based on the delivery of a pool of growth factors and cytokines with tissue healing potential, and has been widely used in sports medicine settings aiming to enhance tissue healing [2]. Both PRP and adult MSCs are physiological means to combat injury. In this context, regenerative medicine seeks to enhance these endogenous resources to help tissue homeostasis prevail over hostile microenvironments.

Regenerative technologies, both allogeneic and autologous, have emerged as an industry, and the potential market is expected to reach US$8 billion by 2020 [3, 4]. Orthopedics and sports medicine are among the areas that will have the greatest applications. Athletes (professional and recreational) seek novel regenerative medicine interventions to heal injuries, and to rapidly resume their desired sports activities. Cell therapies are offered in several stem-cell centers around the world, not only for sports injuries [5], but also to treat devastating illnesses including cerebral palsy, Alzheimer disease, or multiple sclerosis, among others. Medical tourism is an internet-driven business, mediating a rapid expansion of stem-cell clinics, mostly in countries with permissive regulatory conditions [6].

However, stem-cell therapies are still in their first stages of implementation, and much research is still necessary before they can meet the hope that has been put on them. To refrain from pursuing unproven expensive cell therapies, the International Society for Stem Cell Research (ISSCR) offers clear information about disproved efficacy and associated risks (ISSCR, 2013) [7]. Also, a set of performance guidelines is available for responsible administration of stem-cell therapies (ISSCR, 2008) [8].

Aiming to provide an update for healthcare professionals interested in regenerative therapies, we address what is known about the composition of cell-based products (i.e., adult mesenchymal stromal/stem-cell products) and other differentiated cell therapies, their combination with PRP, and the assumptions or paradigms on which to base their mechanisms of action. We also review the current literature about clinical outcomes following the use of cell therapies in sports medicine for tendon and joint conditions.

## 2 Regenerative Medicine Technologies

### 2.1 Regenerative Medicine Products

The three main regenerative medicine injectable products undergoing investigation for tissue healing are (1) most importantly, cells, which drive healing mechanisms; (2) PRP; and (3) conditioned media (CM) from cells (Fig. 1).

In general, cells or other regenerative products for musculoskeletal injuries are delivered locally. This is in contrast to the systemic route of administration in some other pathologies, such as systemic lupus erythematosus [9].

#### 2.1.1 Cellular Products

Cell therapies include a broad range of subtypes, from injectable mixtures of cell populations, as is the case with bone marrow concentrate (BMC), and stromal vascular fraction (SVF), to refined MSC preparations with trilineage differentiation capacity and characteristic surface proteins. Despite these specific features, there are not unequivocal markers of cell quality and functional efficacy in vivo. Furthermore, comparability between trial clinical outcomes can be hindered because of variability in the quality of MSCs associated with fabrication reagents and procedures. Central to progress in the field is a description of manufacturing procedures and the development of products based on standardized parameters. Therefore, for laboratory-expanded cells, authors are encouraged to provide specific in-process data, such as initial yield ($\times 10^6$/days at passage zero (P0), passage 2 (P2) cumulative population doublings, and P2 epitopes (+/−) [10].

As an alternative, tissue-specific biopsies can be harvested and differentiated cells, such as chondrocytes for cartilage, and tenocytes or skin fibroblasts for tendon conditions, are implanted after 3–4 weeks of growth in vitro. Presently, the main interest is focused on injecting cells and PRPs; both are mostly used on an autologous basis to avoid any host immune responses.

Hereinafter, we address the main characteristics of bone marrow and fat-derived regenerative products as well as PRP and CM.

#### 2.1.1.1 Stromal Vascular Fraction and Adipose Stromal/Stem Cells

Adipose tissue can be considered the richest source of stem cells in our body. A simple adipose graft has been injected in joint conditions as an adjuvant to BMC.

---

© Springer
Alternatively, adipose tissue can be fractionated into mature adipocytes, blood, and the SVF (Fig. 2). In 1964, Rodbell [11] isolated SVF for the first time using proteolytic enzymes and centrifugation. SVF can be obtained in a few hours using kits and following commercial protocols, without altering the relevant biological characteristics of the cells. SVF is a fresh product prepared at the point of care but qualifies as an advanced therapy medicinal product (ATMP) because it involves the use of proteolytic enzymes to obtain a cell suspension. This cell suspension is then centrifuged and the cell pellet is termed SVF. Thus, the use of SVF for orthopedic conditions requires Food and Drug Administration (FDA) and institutional review board (IRB) approval.

The injection of these preparations provides the host tissue with a heterogeneous cell population including hematopoietic stem cells, endothelial cells, and adipose-derived stromal/stem cells representing 2–10 % of SVF. CD34+ cells (angiogenic cells) are present in large numbers and could compose up to 63 % of SVF [12, 13]. Overall, CD34+ cells constitute the main part of the stem-cell niche, and may also favorably influence modulation of neovascularization. Vascularization is crucial in early healing mechanisms to provide oxygen and nutrients for the metabolic needs of activated cells but it is downregulated in the later stages of healing.

Alternatively, to improve purity and obtain a larger number of MSCs, SVF can be culture expanded (Fig. 2a). Zuk et al. [14], pioneered the characterization of multipotent stem cells from human fat-derived SVF, currently named ASCs (adipose stromal/stem cells).

The International Society for Cellular Therapy (ISCT) has proposed four criteria for adult mesenchymal-stem-cell characterization: (1) plastic adherence, (2) at least tri-lineage differentiation capabilities, (3) expression of CD73, CD90, and CD105, and (4) lack of expression of CD14, CD11b, CD34, CD45, CD19, CD79, and human leukocyte antigen–antigen D related [14].

Interestingly, the potential of ASCs is independent of the anatomical fat source [15]. However, ASCs from aged people are less proliferative, and can be of lower quality, because of telomere shortening and DNA damage, compromising their clinical success [16].

2.1.1.2 Bone Marrow Concentrates, and Bone-Marrow-Derived Mesenchymal Stromal/Stem Cells Bone marrow aspirates, from the iliac crest or other sites, can be processed (most often by centrifugation) at the point of care to concentrate the nucleated cells of the marrow, which contain various populations of progenitors (Fig. 2b). This ‘fresh’ product obtained through minimal manipulation is named BMC or BMAC, bone marrow concentrate or bone...
marrow aspirate concentrate. Most cells are CD34− heme progenitors, and very few (0.01–0.001%) are multipotent MSCs [17]. A subpopulation that only retains chondrogenic potential has also been identified, making them particularly attractive for joint conditions [18].

Because of the huge interest in using BMC as ‘therapy’, the performance of different commercial systems is compared in terms of cell recovery, stem/progenitor cells (CD34+), and colony-forming units (CFU-F); that is, MSCs [19].

Alternatively, mononuclear cells can be isolated by density gradient centrifugation, and cultured on plastic surfaces to remove hematopoietic mononuclear cells. Adherent MSCs are then expanded for several generations. Since cells are isolated from the niche that controls their phenotype (i.e., other cells, cytokines, extracellular matrix [ECM], molecular forces, and so on), following substantial manipulation, both bone marrow-derived mesenchymal stem cells (BM-MSCs) and ASCs are therefore considered an ATMP as ruled in the EU Directive no. 1394/2007 [20]. Similarly, in the USA, ATMPs are regulated by CBER (the Center for Biologics Evaluation and Research). These treatments are only allowed via an IRB approval protocol.

2.1.1.3 Peripheral Blood Progenitor Cells Peripheral blood progenitor cells (PBPC) are CD34+ cells collected in apheresis procedures typically after treating the patient with a granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony-stimulating factor (GM-CSF) [21]. Currently, PBSC are used as a source of stem cells in the hematopoietic reconstitution. In addition, PBSCs can be harvested and cryopreserved. This product is injected within tendons and in the joint cavity to enhance tissue healing.
2.1.2 Platelet-Rich Plasma Technologies

PRP is a plasma preparation with a number of platelets above the normal level in blood, generally obtained after centrifugation of peripheral blood. PRP contains a complex molecular mixture including signaling and adhesive proteins. At present, it is evident that the therapeutic effect of PRPs in tissue healing is not only attributed to growth factors, but also to a myriad of chemokines and other cytokines actively involved in tissue-repair processes, including cell proliferation, differentiation, migration, angiogenesis, and the synthesis of ECM [22]. Importantly, PRPs also trigger synthesis of neurotrophic and angiogenic factors by local cells, thereby amplifying the initial effects [23].

Combining PRP with cell therapies provides a controlled milieu for cells. In addition, PRPs can function as both cell carriers and cytokine delivery systems. In fact, upon plasmatic fibrinogen cleavage and polymerization by thrombin action, the newly developed fibrin constitutes a suitable adhesive scaffold for cell delivery [24]. Platelets embedded within this fibrin scaffold slowly release the molecular pool stored in the alpha granules as well as other small molecules contained in dense granules [25]. What makes PRP attractive is the delivery system embodied by fibrin that confines molecules and cells to the chosen site. The molecular characterization of PRPs is challenging and varies quantitatively from one individual PRP to another and from one formulation to another [26]. Up to now, it has been impossible to establish quality criteria for PRP products, because there is not enough information about which are the main molecules responsible for the therapeutic effect in each specific tissue condition. Although the initial paradigm of PRP actions was based on platelet number, currently we know that most PRP effects are the consequence of activation of migratory and local cells [27].

More research is necessary to describe how PRP influences the regenerative actions of MSCs. For example, it was recently shown that PRP could favor stemness, and prolongs survival of transplanted cells [28-30]. In addition, PRP can control secretory function [31] in different manners depending on PRP formulation and cell phenotype. Van Pham et al. [32] studied the behavior of the mixture of human ASCs obtained from SVF of ten individuals and expanded with PRP. In addition, ASCs were re-suspended in 3 mL of human PRP, after which the product was implanted into a cartilage injury in immunodeficient mice. This study showed that PRP efficiently stimulated ASC proliferation, and does not change the marker expression of ASCs, but it modifies the expression of SOX-9 (SRY [sex determining region Y]-Box 9), collagen type 2, and aggrecan. Also, PRP reduces vascular endothelial growth factor (VEGF) expression by ASC [32].

2.1.3 Conditioned Media

A less investigated product to be used for regenerative purposes is the conditioned culture media (CM). While growing in vitro, cells release to the extracellular milieu a pool of cytokines, chemokines, growth factors (including transforming growth factors [TGF-α, TGF-β], hepatocyte growth factor [HGF], epidermal growth factor [EGF], fibroblast growth factor [FGF-2], insulin growth factor [IGF-1], VEGF, angiopoietin [ANGPT-1], among others), as well as matricellular proteins, enzymes, microvesicles/exosomes, messenger RNAs (mRNAs), and microRNAs [33] (Fig. 1c). The source of CM is cells cultured in vitro under specific consistent protocols. CM is composed from the soluble molecular components of cell secretome, which can be tailored for specific therapeutic actions. Actually, the therapeutic potential of CM is based on one of the paradigms of MSC actions: the trophic and paracrine effects on local cells.

A major advantage of CM is that it can be easily manufactured, sterilized, packaged, and stored, and thus can constitute an ‘off-the-shelf’ stem-cell product.

The therapeutic value of the stem-cell CM is under research [34, 35]. Numerous patent applications have been filed in recent times (i.e., US2012/0276215A1). For example, an ongoing clinical trial [36] in OA is assessing the safety and feasibility of trophic factors from umbilical cord mesenchymal stem cells.

2.2 The Mechanism of Action of Regenerative Medicine Products

There are a number of conditions in which regenerative medicine products, in particular MSCs, have been investigated, and thousands of articles have been published on this topic. Consistent with the complexity of these products, extensive literature from the past decades indicates that regenerative medicine products modulate almost every facet of repair mechanisms (Fig. 3).

When injected systemically, the assumption that MSCs home to the relevant tissue and replace injured cells was based on both their migratory and differentiation capacities [37, 38]. Site-directed implantation (i.e., cells loaded in collagen membranes [or other scaffolds] placed within chondral or osteochondral defects) can also influence cell fate. In addition to tri-lineage differentiation capacity (i.e., bone, cartilage, and fat), adult MSCs further differentiate to tendon or ligament in the presence of environmental molecular cues including ligament/tendon-derived matrix [39].
endothelial growth factor, HGF hepatocyte growth factor, IDO indoleamine 2,3-dioxynogenase, IFN interferon, IGF insulin growth factor, IL interleukin, MCP monocyte chemotactic protein, MSCs mesenchymal stem cells, PDGF platelet-derived growth factor, PGE2 prostaglandin E2, SDF stromal cell-derived factor, TGF transforming growth factor, TLR toll-like receptor, TNF tumor necrosis factor, TSG-6 TNFα-stimulated gene 6, TNFα-inducible protein 6, VEGF vascular endothelial growth factor

Fig. 3 Mechanism of action of cell therapies: while still unclear, several hypotheses have been proposed. Differentiated cells are injected or implanted within tissue lesions to (1) engraft, synthesize ECM molecules, integrate with the surrounding tissue, and return tissue to homeostasis conditions and full functional capabilities. MSCs can (1) engraft the tissue provided that the conditions of the host tissue are favorable for differentiation; (2) modulate the inflammatory response; (3) provide trophic and antiapoptotic factors; (4) interact with the progenitor niche. ECM extracellular matrix, EGF...
allogeneic MSCs can differentiate into local cells, and can activate the host immune response [62]. The presence of immunogenicity after cell differentiation can decrease their therapeutic effect. Current knowledge supports the theory that MSCs are immune evasive and not immune privileged, an issue that requires further scientific clarification.

Efficacy doses of regenerative medicine products are unknown. Regenerative medicine products are biological response modifiers in contrast to pharmaceutical agents or recombinant proteins. This means that they induce further cellular and molecular changes over time. However, because of their perception as drugs, MSC doses for expanded cells are measured in the millions or billions of cells, and initially calculated based on the recipient’s body weight for systemic or intrathecal routes of administration [63]. Intraleosional delivery compared with systemic administration has the advantage that cells arrive directly at the target tissue, avoiding cell losses that may occur during migration. Doses of PRP are measured as concentration of platelets or multiples of platelets relative to the number in peripheral blood.

### 3 Regenerative Therapies in Clinical Practice

To obtain more precise information about the clinical outcomes following cell therapies, we conducted a narrative review, categorizing the studies included in the review by target tissue and underlying pathology. We excluded studies examining the efficacy of autologous chondrocyte implantation (ACI) because they have been recently reviewed [64]. For the same reason, we did not review PRP studies [2, 65, 66].

#### 3.1 Search Strategy

The review methodology is shown in Fig. 4. Articles were categorized according to condition, and whether the experimental cell product was applied by injection or at surgery. Additionally, studies were categorized according to whether fresh ‘stem-cell-based products’ (same-day procedures) or laboratory-expanded cells for 3–4 weeks were used. Data relating to experimental design, condition, patient population, as well as specific cell product,
intervention type, and clinical outcomes were extracted and tabulated (Tables 1, 2, 3).

3.2 Results

Nine clinical studies used autologous cells obtained from different sources to treat tendon conditions [67–76] (Table 1). In addition, we identified 22 studies in which laboratory-expanded MSCs (n = 11) [77–88] or stem-cell-derived products (n = 11) were injected intra-articularly [89–99] (Table 2). Thirty-two studies performed arthroscopic/surgical delivery of cells [24, 101–133]; nine of these studies used laboratory-expanded cells [100–108] while stem-cell-derived products were used in 23 studies [24, 109–133] (Table 3).

A pioneer study was conducted in Australia by Wang et al. [70, 71], who implanted 2–5 × 10⁶ autologous tenocytes, obtained from patellar tendon biopsies and further expanded in vitro, within the lateral epicondyle by ultrasound-guided injections with no peppering in 20 patients with recalcitrant tendinopathy. No donor-site complication was found at follow-up. Outcome scores included visual analog scale (VAS), decreasing from 5.73

3.2.1 Tendon Conditions

Studies examining the efficacy and safety of cell therapies in tendon conditions are shown in Table 1 [67–76]. Cell therapies consisted of culture-expanded tenocytes [70, 71], or skin fibroblasts [67, 74]; also, BMC [68, 69, 72], SVF [76], and PBPC [73] have been used in five case series. A pioneer study was conducted in Australia by Wang et al. [70, 71], who implanted 2–5 × 10⁶ autologous tenocytes, obtained from patellar tendon biopsies and further expanded in vitro, within the lateral epicondyle by ultrasound-guided injections with no peppering in 20 patients with recalcitrant tendinopathy. No donor-site complication was found at follow-up. Outcome scores included visual analog scale (VAS), decreasing from 5.73

...
Table 2 Conservative management: injections of cell products in joint conditions

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design, condition, N/patient population</th>
<th>Intervention/cell product</th>
<th>Outcome measurements/follow-up/results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection of expanded mesenchymal stem cells (N = 11) [77–88]</td>
<td></td>
<td>Injection, pre-injection of 3–5 mL of 12.5 % dextrose + 0.1 % lidocaine in normal saline into the ACL 2–5 days prior to 694 × 10^6 BM-MSCs/2.7 mL injection</td>
<td>1, 3, 6, and 12 months, VAS decrease 1.7, p = 0.25, LEFS increase 23.3, p = 0.03, self-rated improvement 86.7 %, 7/10 patients showed improvements in at least 4/5 measurements (VAS, LEFS, FRI, subjective % improvement and MRI)</td>
</tr>
<tr>
<td>Centeno et al. (2015) [77]</td>
<td>Case series, ACL tears, N = 10, grade 1, 2, 3 tears</td>
<td>Injection of 5.76 × 10^6 BM-MSCs</td>
<td>11.8 months after cell infiltration improvement of 60 %, whereas 19 % in the control group. VAS reduction 63 % and frequency and duration of pain decreased by 41.7 % and 83.3 %. No complications among treated patients</td>
</tr>
<tr>
<td>Centeno and Freeman (2014) [78]</td>
<td>Controlled study, severe carpometacarpal OA, n = 6 patients treated and n = 10 untreated controls, chronic</td>
<td>Injection of 5.76 × 10^6 BM-MSCs expanded with platelet lysate</td>
<td>At 12 months, 60 % improvement in treatment group (95 % CI 22–99) and 19 % in the control group (95 % CI −110 to 73); p = 0.003, pain decrease, frequency, and duration of pain decreased by 41.7 % and 83.3 %. No complications among treated patients</td>
</tr>
<tr>
<td>Centeno et al. (2011) [79]</td>
<td>Controlled study, knee OA, n = 6 experimental, n = 10 controls. Setting: interventional pain practice</td>
<td>Injection, autologous expanded BM-MSCs 8–9 × 10^6 cells</td>
<td>VAS, walking time to pain improved in ¾ patients, number of stairs they were able to climb improved significantly</td>
</tr>
<tr>
<td>Davatchi et al. (2011) [80]</td>
<td>Case series, knee OA, N = 4</td>
<td>Injection, 20–24 × 10^6 BM-MSC, suspended in saline serum and injected under fluoroscopy</td>
<td>No adverse events, pain, functional status, and walking distance improved at 6 months but the effect slightly decreased at 12 months. MRI showed increase in cartilage thickness, extension of the repair tissue over the subchondral bone and decrease in the size of subchondral edema in 3/6 patients</td>
</tr>
<tr>
<td>Emadedin et al. (2012) [81]</td>
<td>Case series, knee OA, N = 6 females requiring prosthesis</td>
<td>Injection, Ad-MSCs three different doses, 1 × 10^7 cells; 5 × 10^7 cells; 1 × 10^8 cells</td>
<td>Improvement VAS, WOMAC at 6 months, clinical, radiological, arthroscopic, and histological evaluations showed results in the high-dose group. The size of cartilage defect decreased in the medial femoral condyles and tibial plateau on the high-dose group. Hyaline cartilage on histology</td>
</tr>
<tr>
<td>Jo et al. (2014) [82]</td>
<td>Case series, knee OA, N = 18</td>
<td>Injection, MSCs isolated from SVF and expanded. Arthroscopic implantation vs injection</td>
<td>At 28.6 months IKDC and Tegner better in the implantation than injection group. ICRS grade assessed arthroscopically, better in the implantation group, p = 0.041</td>
</tr>
<tr>
<td>Kim et al. (2015) [83]</td>
<td>Cohort study, n = 20 patients treated with MSCs + PRP injection vs n = 20 matched patients implanted arthroscopically with MSCs + PRP</td>
<td>MSCs isolated from SVF and expanded. Arthroscopic implantation vs injection</td>
<td>MRI at 1 year, 27 % decrease of poor cartilage areas, 68–78 % improvement in algofunctional index</td>
</tr>
<tr>
<td>Orozco et al. (2013), (2014, 2-year follow-up) [84, 85]</td>
<td>Case series, knee OA, N = 12</td>
<td>Injection, autologous 40 × 10^6 BM-MSC</td>
<td>Lequesne, WOMAC, VAS, MRI, significant reduction in pain at 6 and 12 months post-treatment. VAS decreased significantly. Total WOMAC decrease at 12 months but not significant. Poor cartilage index used to quantify T2 mapping showed promising outcomes in 74 % of patients at 12 months</td>
</tr>
</tbody>
</table>
### Table 2 continued

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design, condition, N/patient population</th>
<th>Intervention/cell product</th>
<th>Outcome measurements/follow-up/results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vangsness et al.</strong> (2014) [87]</td>
<td>RCT, surgically removed meniscal tissue, N = 55 patients, injections 7–10 days after partial medial meniscectomy</td>
<td>Single knee injection, 50 × 10⁶ allogeneic MSCs, 150 × 10⁶ allogeneic MSCs with HA as vehicle, control group HA injection</td>
<td>No ectopic tissue formation, at 12 months increased meniscal volume (up to 15 %) in 24 % patients in group A (lower dose) and 6 % patients in group B (higher cell dose) ( p = 0.022 ), no patient in the control group. Significant reduction in pain as measured by VAS. Evidence of meniscus regeneration in patients who received cells</td>
</tr>
<tr>
<td><strong>Vega et al.</strong> (2015) [88]</td>
<td>RCT, knee OA severity II–IV K-L, n = 15 per group</td>
<td>Injection/allogeneic 40 × 10⁶ BM-MSCs vs HA (60 mg single dose)</td>
<td>VAS, Lequesne, WOMAC, 6 and 12 months. No differences in pain between groups except for VAS at 12 months, Lequesne and WOMAC decreased only in experimental group. 77 % patients satisfied in the cell group and 38 % in the control group. No serious adverse events. MRI T2 relaxation measurements showed cartilage quality improvement in the MSC group</td>
</tr>
<tr>
<td><strong>Injection of SVF and/or BMC (N = 11) [89–99]</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bui et al. (2014)</strong></td>
<td>Case series, knee, N = 21</td>
<td>SVF + PRP</td>
<td>8.5 months, improvement in VAS, function, and MRI</td>
</tr>
<tr>
<td><strong>Centeno et al. (2014)</strong></td>
<td>Case series, knee OA, N = 424 knees, early stage</td>
<td>Injection BM-MSCs two doses ( n = 185 ) dose ( &gt; 4 \times 10⁶ ) and ( n = 224 ) dose ( &lt; 4 \times 10⁶ )</td>
<td>IKDC, VAS; 1, 3, 6 months and 1 year; better with higher dose, ( p &lt; 0.001 )</td>
</tr>
<tr>
<td><strong>Centeno et al. (2014)</strong></td>
<td>Registry data, knee OA, 840 procedures</td>
<td>Injection, 616 BMC and 224 BMC + adipose</td>
<td>LEFS increase, NPS decrease, addition of adipose tissue no benefit. Adverse events 6 % in BMAC group and 8.9 % in BMC + adipose</td>
</tr>
<tr>
<td><strong>Fodor and Paulseth (2016)</strong></td>
<td>Case series, knee OA (K-L I–III), N = 6 patients, 8 knees</td>
<td>Injection SVF, 14.1 × 10⁶ nucleated cells</td>
<td>3 months and 1 year improvement in WOMAC and VAS. MRI at 3 months, no detectable differences</td>
</tr>
<tr>
<td><strong>Gibbs et al. (2015)</strong></td>
<td>Case series, knee OA, N = 4 patients, N = 7 joints</td>
<td>Injection, SVF + PRP</td>
<td>2, 3, 6, 8, and 12 months, KOOS, get up and go test, stair climbing test returned to normal</td>
</tr>
<tr>
<td><strong>Kim et al. (2014)</strong></td>
<td>Case series, knee OA K-L I–IV, N = 41 patients (75 knees)</td>
<td>Injection, BMC + fat</td>
<td>3, 6, 12 months. Significant improvement in VAS, IKDC, SF-36, more effective in early OA</td>
</tr>
<tr>
<td><strong>Koh et al. (2013)</strong></td>
<td>Case series, knee OA, N = 18</td>
<td>SVF from infrapatellar pad injected (1.18 × 10⁶) with PRP, two injections PRP 1.28 × 10⁶ platelet/µL</td>
<td>3 months, 1 and 2 years, WOMAC, Lysholm, VAS. Improvement in MRI and clinical scores. Improvement related to number of injected cells</td>
</tr>
<tr>
<td><strong>Koh and Choi (2012)</strong></td>
<td>Knee cartilage defects, retrospective comparative study</td>
<td>SVF + PRP versus PRP (SVF is from infrapatellar pad)</td>
<td>16.4 months VAS, Lysholm, Tegner better in SVF + PRP</td>
</tr>
<tr>
<td><strong>Oliver et al. (2015)</strong></td>
<td>Case series, knee OA, N = 70 patients, K-L: II–IV</td>
<td>3 mL BMC + 2 ml liposapirate in 0.00125 % lidocaine</td>
<td>3 and 6 months, KOOS sub-scores improved but not significantly</td>
</tr>
<tr>
<td><strong>Pak et al. (2013)</strong></td>
<td>Chondromalacia patellae, N = 3</td>
<td>SVF + PRP</td>
<td>3 months, VAS, FRI, ROM, MRI pain and function improvement, and MRI evidence of cartilage regeneration</td>
</tr>
</tbody>
</table>
Table 2 continued

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design, condition, N/patient population</th>
<th>Intervention/cell product</th>
<th>Outcome measurements/follow-up/results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pak et al. (2013)</td>
<td>Case series, OA, N = 91 patients. Hip n = 22; knee n = 74; ankle n = 2; low back n = 2</td>
<td>SVF + HA + activated PRP + three injections of activated PRP weekly within a month</td>
<td>Mean follow-up 26 months, significant decrease in VAS at 1 and 3 months. Pain and swelling 1 day after SVF + HA + activated PRP in 37% of patients. Safety assessment: infection 0%, tumor 0%, tenosynovitis/tendonitis 22%, neurologic 1% hemorrhagic stroke 2 weeks after the procedure</td>
</tr>
</tbody>
</table>

ACL anterior cruciate ligament. Ad-MSCs adipose-derived mesenchymal stem cells. BMAC bone marrow aspirate concentrate, BM-MSCs bone marrow-derived mesenchymal stem cells, BMC bone marrow concentrate. CI confidence interval. FRI Functional Rating Index, HA hyaluronic acid, ICRS International Cartilage Research Society, IKDC International Knee Documentation Committee, K-L Kellgren-Lawrence score, KOOS Knee injury and Osteoarthritis Outcome Score, LEFS Lower Extremity Functional Scale, MRI magnetic resonance imaging, MSC mesenchymal stem cell, NPS Numeric Pain Scale, OA osteoarthritis, PRP platelet-rich plasma, RCT randomized controlled trial, ROM range of movement, SVF stromal vascular fraction, VAS visual analog scale, WOMAC Western Ontario and McMaster Universities Osteoarthritis Index.
<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design, condition, N/patient population</th>
<th>Intervention/cell product</th>
<th>Outcome measurements/follow-up/results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akgun et al. (2015) [100]</td>
<td>Prospective randomized pilot study, N = 14, knee, full thickness chondral lesions &gt;2 cm²</td>
<td>Mini-arthroscopy m-AMI vs m-ACI. MSCs harvested from synovia and expanded/chondrocytes harvested and expanded</td>
<td>6, 12, and 24 months, m-AMI group better for all KOOS sub-scales than m-ACI (pain, symptoms, ADL, sport/rec). MRI at 24 months m-AMI excellent and m-ACI group good. Bone marrow edema signal decreased to normal in m-AMI. No complications</td>
</tr>
<tr>
<td>Haleem et al. (2010) [101]</td>
<td>Case series, knee chondral lesions N = 5 patients full-thickness defects, 3–10 cm²</td>
<td>Arthroscopy 15 × 10⁶ BM-MSCs + PR-fibrin glue (7.7 × 10⁶ pts/mL) 2 × 10⁶ cells/cm² covered with periosteal flap</td>
<td>All patients improved ICRS score at 6, 12 months, second look arthroscopy in 2 patients showed nearly normal joints; MRI of 3 patients showed complete defect fill, 2 patients showed incomplete congruity</td>
</tr>
<tr>
<td>Koh et al. (2014) [102]</td>
<td>Case series, N = 37 knees, chondral lesions 2.3–8.9 cm²</td>
<td>2.5–6.1 × 10⁶ MSCs from SVF</td>
<td>Improved IKDC, Tegner. ICRS grading at 2nd look 76% of knees rated abnormal</td>
</tr>
<tr>
<td>Lee et al. (2012) [103]</td>
<td>Prospective cohort study, knee chondral lesion N = 35 patients arthroscopic microfracture + outpatient injection of 10⁷ BM-MSCs + 2 mL HA versus N = 35 MSCs implanted in open surgery</td>
<td>VAS, IKDC, Lysholm, significant improvements in both groups at 24 months</td>
<td></td>
</tr>
<tr>
<td>Nejadnik et al. (2010) [104]</td>
<td>Observational cohort study, knee chondral lesions, N = 72</td>
<td>BM-derived MSCs and chondrocyte expansion</td>
<td>No differences between groups</td>
</tr>
<tr>
<td>Saw et al. (2013) [105]</td>
<td>RCT, knee, grade 3–4 chondral lesions N = 50 patients, 25 per group</td>
<td>Subchondral drilling and 5 injections of HA vs PBPC + HA (once per week) at serial visits post-surgery 6 months after surgery 1 injection per week for 3 weeks</td>
<td>No differences between groups at 24-month IKDC. No notable adverse effects. 16 patients per group had histology and 2nd look arthroscopy ICRS histologic score, MRI scans at 18 months, significantly better in the experimental group</td>
</tr>
<tr>
<td>Sekiya et al. (2015) [106]</td>
<td>Case series, knee, single cartilage lesion of the femoral condyle, N = 10 (5 patients underwent concomitant ACL reconstruction among whom 2 had meniscus suturing)</td>
<td>Expanded synovial MSCs with autologous serum for 14 days, arthroscopic implantation on the defect</td>
<td>MRI improved from 1.0 ± 0.3 to 5.0 ± 0.7 p = 0.005, histology performed on 2nd look arthroscopy on 4 patients showed hyaline cartilage in 3 patients and fibrous cartilage in 1 patient, Lysholm no change, Tegner 76 ± 7 before and 95 ± 3 after. Average follow-up 52 months</td>
</tr>
<tr>
<td>Teo et al. (2012) [107]</td>
<td>Case series, patellar OOC, adolescent patients, mean age 16.8 years</td>
<td>N = 20 autologous chondrocytes, N = 3 patients cultured BM-MSC</td>
<td>6, 12, and 24 months. Mean IKDC score, Tegner-Lysholm outcomes, and Lysholm-Gillquist scale improved from 45, 2.5, and 50, respectively at surgery to 75, 4, and 70, respectively, at 24-month follow-up. Complications include periosteal hypertrophy observed in 2 patients</td>
</tr>
<tr>
<td>Wong et al. (2013) [108]</td>
<td>RCT, unicompartmental knee OA and genu varum, n = 28 patients cells + HA vs n = 28 controls</td>
<td>HTO + microfracture + BM-MSCs (injected post-op) vs HTO + microfracture. Expanded BM-MSCs were injected after 22 days</td>
<td>MOCArt adjusted by age, at 1 year better in the cell group. Tegner, Lysholm, IKDC cell treatment added improvement</td>
</tr>
<tr>
<td>Buda et al. (2016) [109]</td>
<td>Case series, osteochondral lesions of the talus and ankle OA, N = 56</td>
<td>BMC + PRF, arthroscopic or open-field surgery, bone filling with demineralized bone matrix in selected cases</td>
<td>12, 24, and 36 months’ follow-up, clinical improvement assessed by AOFAS at 3 time points. MRI in 22 patients, MOCArt revealed complete degree of filling with bone edema in most patients. 16/56 required another intervention (failures) another treatment</td>
</tr>
</tbody>
</table>
### Table 3 continued

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design, condition, N/patient population</th>
<th>Intervention/cell product</th>
<th>Outcome measurements/follow-up/results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buda et al. (2015) [110]</td>
<td>RCT, ankle, osteochondral lesions of the talus, N = 80, 40 per group</td>
<td>ACI vs BMC + PRP arthroscopic debridement</td>
<td>48 months MRI, MOCART similar in both groups, clinical outcomes similar in both groups</td>
</tr>
<tr>
<td>Buda et al. (2015) [111]</td>
<td>Case series, ankle, N = 64 patients, post-traumatic type II focal lesions talar dome</td>
<td>BMC + [porcine collagen powder + PRP] or BMC + [HA membrane + PRP]</td>
<td>6, 12, 18, and 24 months, final follow-up 72 months, AOFAS improvement no differences between both scaffolds. 60/64 patients participate in low-impact sport at 4.8 months and 49/64 patients in high-impact sport at 11.9 months</td>
</tr>
<tr>
<td>Buda et al. (2014) [112]</td>
<td>Case series, knee, hemophiliac patients, N = 5</td>
<td>BMC transplantation, synovectomy, arthroscopic debridement</td>
<td>6, 12, 18, 24, 36 months, IKDC, KOOS significant improvement. MRI, MOCART score (6 and 12 months) showed association with KOOS score, regeneration of subchondral bone and cartilage, histology in 2 patients showed type II collagen and proteoglycans</td>
</tr>
<tr>
<td>Buda et al. (2010, 2013) [113, 114]</td>
<td>Case series, knee osteochondral lesions, N = 30 patients, 28 with post-traumatic lesions, Grade II-IV and 2 patients with osteochondritis dissecans</td>
<td>BMC, concentrated from 60 to 6 mL soaked into HA membrane, implanted arthroscopically and covered with PRP</td>
<td>12 months, macroscopic assessment, all repairs appear almost normal. Clinical improvement. No adverse effects</td>
</tr>
<tr>
<td>Enea et al. (2015) [115]</td>
<td>Case series, knee, focal chondral lesions, 2.5 cm²</td>
<td>Microfracture with collagen immersed in BMC</td>
<td></td>
</tr>
<tr>
<td>Giannini et al. (2009, 2013) [116, 117]</td>
<td>Case series, osteochondral lesions of the talus, N = 49 patients</td>
<td>BMC + scaffold</td>
<td>6, 12, and 4 years AOFAS improved and correlated with MRI findings</td>
</tr>
<tr>
<td>Gobbi et al. (2011) [118]</td>
<td>Case series, knee, grade IV chondral lesions, size 9.2 cm², N = 15 patients, 6/15 had multiple chondral lesions</td>
<td>BMC + collagen I/III matrix</td>
<td></td>
</tr>
<tr>
<td>Kasemkijwattana et al. (2011) [119]</td>
<td>Case series, N = 2, grade 3–4 ICRS classification</td>
<td>Arthroscopic implantation BM-MSCs + 3D collagen</td>
<td>30 months, no complications, significant improvement KOOS, IKDC. Defect fill, stiffness and incorporation to adjacent cartilage as assessed in arthroscopy</td>
</tr>
<tr>
<td>Kim et al. (2015) [24]</td>
<td>Retrospective cohort study, knee N = 54 patients (56 knees) isolated full thickness cartilage lesions K-L 1–2</td>
<td>37 patients (39 knees) SVF without scaffold. 17 patients = knees, SVF + fibrin glue</td>
<td>28.6 months IKDC and Tegner improved, no differences between groups. At 2nd look arthroscopy, better ICRS grade in group 2 Significant differences in clinical outcomes among the age and lesion size group. Age &gt;60 years and lesion size &gt;6 cm² showed poor outcomes</td>
</tr>
<tr>
<td>Kim et al. (2015) [120]</td>
<td>Retrospective case series, knee N = 49 patients (55 knees) isolated full thickness cartilage lesions K-L 1–2</td>
<td>SVF</td>
<td></td>
</tr>
<tr>
<td>Kim et al. (2016) [121]</td>
<td>Case series, isolated knee chondral lesions, K-L grade 1–2, N = 24 patients</td>
<td>Arthroscopic debridement SVF + fibrin glue</td>
<td>24 months, IKDC and Tegner significant improvement, MRI MOCART significant improvement</td>
</tr>
<tr>
<td>Kim et al. (2013) [122]</td>
<td>Case series, N = 45 patients &gt;50 years ankle, osteochondral lesions of the talus</td>
<td>Arthroscopic marrow stimulation, N = 35 patients (37 ankles) vs BMC injection + arthroscopic marrow stimulation</td>
<td>21.8 months, Roles and Maudsley better without BMC, Tegner better in BMC group</td>
</tr>
<tr>
<td>Kim et al. (2014) [123]</td>
<td>Cohort study, N = 49 patients, 50 ankles, osteochondral lesions of the talus</td>
<td>Arthroscopy N = 26 OC stimulation, N = 24 OC stimulation and SVF</td>
<td>All clinical outcomes (VAS, AOFAS, Tegner, MOCART) improved in MSC group compared with controls</td>
</tr>
</tbody>
</table>
**Table 3 continued**

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Study design, condition, N/patient population</th>
<th>Intervention/cell product</th>
<th>Outcome measurements/follow-up/results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koh et al. (2016) [124]</td>
<td>Prospective randomized trial ICRS grade III/IV, symptomatic cartilage defect &gt;3 cm² on the femoral condyle</td>
<td>ADSC + fibrin glue + microfracture vs microfracture</td>
<td>MRI at 24 months, complete cartilage coverage in 65 % of experimental group vs 45 % in the control group. KOOS better in experimental group but no differences in subscores, 2nd look arthroscopy and histology showed no differences between groups</td>
</tr>
<tr>
<td>Koh et al. (2015) [125]</td>
<td>Case series, knee chondral lesions N = 30 patients &gt;65 years</td>
<td>Arthroscopic lavage and injection of 4.2 × 10⁷ SVF cells with 3 mL PRP</td>
<td>2 years, VAS and function improvement, 16/30 patients followed 2nd look arthroscopy: 3 knees normal cartilage, 7 new cartilage partially covered the defect, 4 knees uncertain change, and 2 knees failed healing. 5/30 worsened K-L degree at 2 years</td>
</tr>
<tr>
<td>Koh et al. (2014) [126]</td>
<td>Prospective comparative study, knee surgery HTO PRP vs HTO</td>
<td>Surgery HTO + PRP vs HTO + PRP + MSC</td>
<td>Lysholm, KOOS, VAS. Greater improvement in VAS and KOOS for pain and symptoms in the MSC + PRP group. Arthroscopic evaluation showed fibrocartilage coverage in 50 % of MSCs + PRP and 10 % of the patients in PRP group</td>
</tr>
<tr>
<td>Krych et al. (2016) [127]</td>
<td>Cohort study, knee, full thickness cartilage defects N = 46</td>
<td>Scaffold + BMAC, N = 12; scaffold + PRP, N = 23; scaffold control N = 11</td>
<td>12 months, MRI scaffold + BMAC improved maturation with greater fill and T2 values closer to hyaline cartilage</td>
</tr>
<tr>
<td>Michalek et al. (2015) [128]</td>
<td>Case series, multicenter, osteoarthritis, 1128 patients. 1856 joints (hips and knees), 503 patients candidates for total joint arthroplasty</td>
<td>SVF + PRP</td>
<td>3, 6, 12 months KOOS/HOOS. 80.6 and 91 % of patients improved &gt;50 % at 3-month and 12-month follow-up. 4/503 patients required total hip replacement</td>
</tr>
<tr>
<td>Saw et al. (2015) [129]</td>
<td>Case series, knee, N = 8 patients, ICRS Grade 4 lesions</td>
<td>Open wedge HTO and 5 weekly injections of PBSCs + HA (8:2), 3 additional injections at intervals 6, 12, and 18 months</td>
<td>Histology at 2nd look arthroscopy, ICRS visual assessment scale mean 1274 (1340 is normal cartilage). No infections</td>
</tr>
<tr>
<td>Skowron´ski et al. (2013) [130]</td>
<td>Case series, ICRS grade III–IV cartilage lesions in the knee, N = 54 patients</td>
<td>BMC + collagen</td>
<td>1 and 5 years, Lysholm, KOOS, VAS. At 1 year, 25 points improvement in KOOS and 35 points in Lysholm</td>
</tr>
<tr>
<td>Silva et al. (2014) [131]</td>
<td>RCT, tendon-bone attachment, ACL reconstruction, n = 20 experimental group, n = 23 control group</td>
<td>Arthroscopy, ACL reconstruction, 3 mL BMC, 1.5 mL injected in the graft and 1.5 mL injected within the bone tunnel, without irrigation</td>
<td>MRI at 3 months, no differences between groups in SNR in the upper and lower interzones</td>
</tr>
<tr>
<td>Wakitani et al. (2002, 2011) [132, 133]</td>
<td>Case series, knee, N = 41 patients, 45 knees, cell transplantation performed in 1988</td>
<td>BM-MSC</td>
<td>Safety; 11 years and 5 months follow-up, no tumors, no infections</td>
</tr>
</tbody>
</table>

3.2.2 Joint Conditions

Peeters et al. [136] assessed the safety of intra-articular delivery of MSC products either by injection or surgical implantation. They analyzed 844 interventions, and reported two related adverse events, one infection and one pulmonary embolism, and two non-related adverse events, one prostate cancer and one schwannoma. Minor events included pain, swelling, and dehydration. Most clinical studies do not refer explicitly to adverse events, but when these were considered, minor self-resolving adverse events were reported. Follow-up as long as 11 years has been maintained in one study including 41 patients (45 knees) treated with BMC, and no tumors have been reported [132, 133]. Moreover, a multicenter analysis of adverse events in 2372 patients undergoing adult autologous stem-cell therapy corroborated the safety of MSC-based therapies [137].

We have classified clinical studies into needle injection delivery (Table 2), or surgical/arthroscopic delivery (Table 3). In addition, we have grouped studies according to regulatory criteria as expanded MSCs (i.e., more than minimal manipulation), ‘advanced therapy’ or fresh cell products, interventions performed on the same day (i.e., the day of harvest from the donor), and cell dose. Based on such criteria, there is interest in freshly isolated mixed-cell populations (BMC, SVF), and culture-expanded mesenchymal stromal/stem cells.

3.2.2.1 Needle Injection Delivery

3.2.2.1.1 Intra-Articular Injections of Culture-Expanded Mesenchymal Stem Cells. Anterior Cruciate Ligament

Intraligamentous injection of BM-MSCs improved the integrity of anterior cruciate ligament (ACL) with grade 1–3 tears in seven of ten patients, as determined by analysis of images using Image J software [77].

Meniscus An RCT by Vangsness et al. [87] reported the safety and efficacy of two doses of cells: 50 and 150 million allogeneic BM-MSCs suspended in hyaluronic acid (HA), and injected intra-articularly 7–10 days following meniscectomy. According to outcome data, surgically removed meniscal tissue was regenerated, cartilage surface protected, and joint damage decreased. The number of patients with increased meniscal volume was five of 54 patients (four from the group that received the lower dose of cells, and one from the higher dose group) at 12 months post-procedure. Only three patients maintained the increased meniscal volume at 2 years (mean percentage 18; 95 % CI 4.0–45.6). Other ongoing trials are examining the efficacy of autologous MSCs in meniscus injury grade 3 [138], and also autologous BMC injection in patients undergoing partial or complete meniscectomy [139].

Cartilage Lesions and Osteoarthritis The needle injection technique (intra-articular cell injection) was used in 22 studies [77–99] (Table 2). Eleven studies examined the effect of intra-articular injections of in vitro expanded MSCs. Cell doses ranged from $5.76 \times 10^6$ to $400 \times 10^6$ cells.

Both RCTs [87, 88] used donor-derived (allogeneic) BM-MSCs and used the cell vehicle, HA, as control. Vega et al. [88] included 30 patients who were randomized to either allogeneic BM-MSCs ($n = 15$), or a single high-molecular weight HA injection ($n = 15$). Overall, all case series that evaluated injections of culture-expanded MSCs involved few patients and showed moderately good results, with no safety problems.

3.2.2.1.2 Needle Injection Delivery of Stromal Vascular Fraction or Bone Marrow Concentrate. Freshly prepared SVF combined with PRP was injected in 113 knees, 22 hips, two ankles and two lower backs, and outcomes reported in five cases series [89, 93, 95, 96, 98], and the combination PRP + HA in one study [99]. In addition, one registry data study [91] reported outcomes after 840 knee injections, 616 knees injected with BMC, and 224 knees with a mixture of BMC and HA. The addition of fat to BMC protected, and joint damage decreased. The number of patients undergoing bilateral versus unilateral procedures. Kim et al. also injected BMC and fat in...
### Table 4 Summary of advantages and disadvantages of the different types of cells being examined in clinical studies

<table>
<thead>
<tr>
<th>Cell-based product</th>
<th>Tissue source</th>
<th>Procedure</th>
<th>Regulation</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>IDEAL framework</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenocytes</td>
<td>Tendon</td>
<td>Tissue biopsy</td>
<td>ATMP</td>
<td>Easy and minimally invasive access. Very proliferative cells</td>
<td>Limited information about functionality of transplanted cells. High cost</td>
<td>2a</td>
</tr>
<tr>
<td>Dermal fibroblasts</td>
<td>Skin</td>
<td>Tissue biopsy</td>
<td>ATMP</td>
<td>Easy and minimally invasive access. Very proliferative cells</td>
<td>No information about phenotypic changes towards tenocytic lineage. High cost</td>
<td>2a</td>
</tr>
<tr>
<td>Chondrocytes</td>
<td>Cartilage</td>
<td>Tissue biopsy</td>
<td>ATMP</td>
<td>FDA approved. Carticel® commercially available</td>
<td>Unavailability of tissue. Low yield. Dedifferentiation. High cost</td>
<td>4</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone marrow</td>
<td>Aspiration</td>
<td>Tissue</td>
<td>Delivery of nucleated cells (minimally manipulated). Point of care processing technology same day</td>
<td>Heterogeneous product. Low MSC number. Better results if matrix supported and arthroscopic implantation</td>
<td>2b</td>
</tr>
<tr>
<td>SVF</td>
<td>Adipose tissue</td>
<td>Lipoaspiration</td>
<td>ATMP</td>
<td>Fresh product. Point of care processing technology same-day procedure</td>
<td>Heterogeneous product. Limited understanding of mechanism of action</td>
<td>2d</td>
</tr>
<tr>
<td>Fat graft</td>
<td>Adipose tissue</td>
<td>Tissue harvest</td>
<td>Tissue</td>
<td>Used to augment BMC tissue graft prepared at point of care</td>
<td>Inflammatory effects when mixed with BMC</td>
<td>2a</td>
</tr>
<tr>
<td>PBPC</td>
<td>Peripheral blood</td>
<td>G-CSF or GM-CSF treatment</td>
<td>Blood product</td>
<td>Can be harvested and cryopreserved</td>
<td>Very few studies. Insufficient information on mechanism of action</td>
<td>2a</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>Bone marrow</td>
<td>Aspiration</td>
<td>ATMP</td>
<td>Can be cryopreserved. Allogeneic or autologous. Well characterized</td>
<td>Age-related changes in cells lead to regenerative decline. BM-MSC yield depends on harvesting procedure. High cost. Ectopic bone formation in tendon</td>
<td>2a</td>
</tr>
<tr>
<td>ASCs</td>
<td>Adipose tissue</td>
<td>Lipoaspiration</td>
<td>ATMP</td>
<td>MSC potentiality independent of the harvest site. Can be cryopreserved</td>
<td>Different cell sub-populations. High cost</td>
<td>2a</td>
</tr>
</tbody>
</table>

**IDEAL framework** [136]: stage 1, idea; 2a development, small number of reports; 2b exploration, increased number of reports and patients per report, registries; 3 assessments, RCTs, multicenter studies, analysis of large data sets

ASCs adipose stem cells, ATMP Advanced Therapy Medicinal Product, BMC bone marrow concentrate, BM-MSCs bone marrow-derived mesenchymal stem cells, FDA Food and Drug Administration, G-CSF granulocyte colony-stimulating factor, GM-CSF granulocyte-macrophage colony-stimulating factor, MSCs mesenchymal stem cells, PBPC peripheral blood precursor cells, RCT randomized clinical trial, SVF stromal vascular fraction

---

621 knees, and reported better outcomes in patients with early OA [94]. Pak et al. [99] reported on 100 joints (74 knees, 22 hips, 2 lower backs, and 2 ankles) in 91 patients injected with SVF (10 mL) combined with PRP (2 mL buffy coat) + HA (1 mL 0.5%). Patients returned for four additional PRP injections (freshly prepared, one injection per week), and the treatment lasted 1 month. Patients were followed for up to 30 months (by phone). VAS improved significantly at 1 and 3 months after treatment. Complications included pain and swelling secondary to injection in 37 % of joints and 22 % of patients reported tendonitis/tenosynovitis; no infections were reported. One patient suffered a hemorrhagic stroke 2 weeks after the procedure, but this was considered not related to the procedure, as the frequency of this event in the general population is 1 %. No tumors were found.

---

Gibbs et al. [93] treated four patients (seven joints) with SVF and PRP, followed by rehabilitation for 4 months. Pain and quality of life, as measured using KOOS, improved significantly; mobility returned to normal.
As an alternative to bone marrow and subcutaneous fat, Koh et al. [95] used the infrapatellar pad as a source for MSCs and injected a mean of 1.18 × 10^6 cells with 3 mL of PRP; patients experienced a significant reduction in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (from 49.9 to 30), an improvement in Lysholm score, and significant pain reduction (VAS). Improvements in MRI scores were also reported (p < 0.001).

3.2.2.2 Surgical/Arthroscopic Delivery

3.2.2.2.1 Culture-Expanded Mesenchymal Stem Cells. Tendon–bone healing following ACL reconstruction remains an unresolved issue of ACL surgery. Filling the bone tunnels with PRP does not speed up tendon-to-bone integration [140]. Recently, a randomized trial was performed to assess the suitability of fresh BMC in tendon–bone healing [131]. However, there were no differences between cell-treated patients and controls, as assessed by MRI.

Ten studies described the implantation of culture-expanded MSCs during arthroscopy or open surgery for chondral lesions or OA [96, 100–108] (Table 3). Interestingly, Kim et al. examined the potential differences between arthroscopic implantation of cells, and site-directed delivery by injection: arthroscopic implantation was more accurate and produced better clinical and imaging results [83].

Intra-articular delivery of MSCs with arthroscopic procedures was performed in debridement, microfractures. [103], management of patellar osteochondritis dissecans (OCD) [107], or microfracture with high tibial osteotomy [108]. MSCs were in some studies just implanted in the defect using periosteal flaps or embedded in scaffolds such as collagen [112], HA [103, 105], PRP-fibrin [102], or fibrin glue [24].

One controlled study [100] used ACI as control for AMI (autologous mesenchymal stem cell implantation), with better results in the AMI group. In five case series, joints were treated with synovium-derived MSCs [106], infrapatellar-derived MSCs [96], and BM-MSCs [101, 107, 108].

3.2.2.2.2 Stem-Cell-Based Products: Arthroscopic Delivery of Stromal Vascular Fraction, Bone Marrow Concentrate or Peripheral Blood Progenitor Cells. Surgical implantation of non-expanded cells has been performed in 23 studies (Table 3). Of these, seven studies involved osteochondral lesions of the talus [109, 110, 112, 116, 117, 122, 123] and seven studies used SVF [24, 120, 121, 123–125, 128]; another study [129] used PBPCs and the other 15 studies used BMC [109–119, 122, 126, 127, 130–133]. In several studies, cells were used as an adjuvant to surgical interventions including microfracture or subchondral drilling [115, 122–124], arthroscopic debridement with or without synovectomy [110, 111, 121], arthroscopic lavage [125], and open-wedge high tibial osteotomy [126, 129].

Histology and second-look arthroscopies after cell intervention have been evaluated in several studies [125, 126, 141]. Koh et al. examined 16 knees after arthroscopic lavage combined with injection of SVF [125]. On second look, three knees were considered very positive (normal cartilage appearance), seven were rated positive (new cartilage partially covered the defect), four knees were rated neutral (uncertain change), and two patients experienced failed healing. Moreover, 37 knees were examined after MSC intervention by the same authors, and were evaluated using International Cartilage Repair Society (ICRS) grading. Results showed that 2/37 were normal, 7/37 were nearly normal (grade II), 20/37 were abnormal (graded III), and 8/37 were severely abnormal (graded IV). High bone mass index (BMI) and large lesions were predictors of poor clinical outcomes. The same authors [126] evaluated at second look (median 19.8 months post-surgery) patients that followed high tibial osteotomy (HTO) with SVF + PRP or HTO + PRP, and results revealed that patients in the SVF + PRP group showed more regenerative changes as assessed by the Kanamia grading system [126]. In four of the five patients treated with 7–8 mL of PBPC + 2 mL HA, Saw et al. [141] found regenerated cartilage integrated with surrounding tissue. Histology showed predominance of type II collagen, particularly in deeper layers with collagen type I in the superficial layer. These findings, together with the columnar morphology of cells, could reveal features of hyaline cartilage [141]. However, to date, control of the fate of adult MSCs within the joint is far below expectations, with the regenerated tissue having features of fibrocartilage and a lack of architectural organization [142].

4 Conclusions

This review of the recent literature indicates that several different cell phenotypes, including tendon, skin, or mesenchymal stem cells, can be suitable for tendon conditions, with applications performed most often with ultrasound-guided percutaneous injections. Indeed, the complexity of articular conditions has shifted research from autologous chondrocyte implantation to the use of a variety of mesenchymal stromal cell products. MSCs are applied not only for the treatment of chondral defects, but also for those of ligaments and the meniscus, and to reduce joint degeneration and reduce the burden of OA. Cells can engraft joint tissues especially at lesion sites. However, multipotency...
may not be the major determinant of success, and repair may rely on a combination of differentiation ability and paracrine effects able to stimulate the ability of exogenous cells to promote endogenous healing mechanisms. A moderate amount of basic science work shows that the approach may work, and a sizeable amount of animal work shows that MSCs and other biological interventions seem to have some effect. However, translational work in human medicine is lacking, and the small volume of well-performed work in humans shows that essentially these therapies, though based on sound basic science findings, do not seem to work particularly well for clinical purposes.

It is likely that opportunities for developing effective treatment for musculoskeletal tissues will arise only after the secrets of MSCs have been unveiled. When transplanting cells, the conditions of the host tissue are of high functional importance as differentiation into suitable cell phenotypes can be inhibited by inflammatory factors produced by the host tissue/organ. The anti-inflammatory properties of PRP can help prevent environmental hostility.

Numerous hurdles need to be overcome as cell therapy progresses. On the one hand, technical challenges associated with robust cell manufacturing at reduced costs are mandatory. On the other hand, use of these technologies will require identifying and understanding the heterogeneity of stem-cell products as well as specific features of disease stage and progression. In this context, identification of biomarkers can serve not only to assess changes in tissue quality, but also to subgroup patients and tailor biological interventions according to specific pathological processes.

Compliance with Ethical Standards

Funding No sources of funding were used to assist in the preparation of this article.

Conflict of interest Nicola Maffulli and Isabel Andia declare that they have no conflicts of interest relevant to the content of this review.

References

isS6odBg9QHcsGe9whHw5lWHWq1wXmNvTCAFodHRwczo
898
899
02003131&rank=1
896
923
922
921
86. Akgun I, Unlu MC, Erdal OA, et al. Matrix-induced autologous mesenchymal stem cell implantation versus matrix-induced


