The Role of Stem Cells in Surgical Repair

13

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Introduction

The developing human body is created, or rather replicated and differentiated, from a single cell. Initially this single cell is comprised of two haploids (half-cells) that unite to create one single cell or "embryo." Development of the human body from this single, immature, pluripotent cell, or stem cell, involves a sophisticated process of cell division, cell differentiation, and inter-cell signaling. This stem cell progresses into a multi-organ body comprised of many tissue types and cell lines. Some cells in our body maintain some abilities of these immature cells and are also referred to as stem cells.

Currently, there are three general domains of stem cells: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells. It is generally considered that most adult stem cells are multipotent, i.e., they can differentiate only toward end-stage cell lines of the germ layer from which they derived. One exception is the mobilized peripheral blood stem cell which has been found to be pluripotent in animal study [1, 2]. ESCs and iPSCs are considered pluripotent, i.e., they can differentiate to end-stage lines of all three germ layers. ESCs are derived from embryonal tissue, have ethical concerns, and have fallen onto the back burner of stem cell research. Induced pluripotent stem cells are derived through the genetic manipulation of somatic cells, have safety concerns, and are under further benchtop development. Adult stem cells can be harvested from multiple human tissues, have been fully developed on the benchtop but require clinical translation with efficacy studies, and have therefore become the current major focus of research clinicians. Since the majority of regenerative cartilage research and development has focused on adult stem cells, it will be the focus of this chapter.

There are four stem capabilities which differentiate these cells from other cells in our body: the ability to self-renew, the ability to differentiate into distinctive end-stage cell types, the ability to monitor and respond to environmental change, and the ability to release a number of molecules to affect their environment [3]. There are cells with stem capability in many tissues including adipose tissue, the synovium of joints, the superficial and deep layers of cartilage, the blood, tendon tissue, and muscle tissue. While initial in vitro study of these cells focused on their ability to divide and differentiate, recent animal and human studies have investigated the natural function of these cells in vivo. It is now clear that some adult stem cells have the ability to monitor their local and the systemic environment for stimuli, mobilize locally and/or systemically

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in settings of environmental insult, interact with their surrounding environment through paracrine effects, and differentiate to an end-stage cell if necessary [1, 2, 4–9] (Fig. 13.1). Stem cells can release a broad spectrum of macromolecules through secretory vessels, sometimes called exosomes or secretomes, which may contain proteins, chemokines, cytokines, and messenger RNA with trophic, chemotactic, and immunomodulatory potential depending upon the environmental stimuli [11]. Through these secretomes, which have paracrine cellular effects, or through differentiation, they participate in injury response, tissue healing, and tissue regeneration [9]. Considering these properties, it is clear that these cells are innate to the body's maintenance, repair, and stress response systems.

Cell Sources and Processing Considerations

In an attempt to harvest the properties of stem cells, researchers and clinicians have studied tissues from different sources and preparation processes. It is important to delineate concentrated and/or simple processed tissues from culture-expanded tissues, as these methods produce different cell numbers. For example, adult bone marrow contains plasma, red blood cells, platelets, red blood cell/platelet precursors, and other nucleated cells. Through centrifugation and selective harvest, one can obtain a fraction of bone marrow which has nucleated cells with stem capabilities, with quantitative studies suggesting 30–317,400 cells/mL of bone marrow are available [12]. Additionally, upon culture of a fraction of bone marrow, selection of the plastic-adherent cells after culture, and further culture of these adherent cells, one can obtain an even greater number of stem cells. The cells obtained from the culture process from bone marrow have been termed "bone marrow-derived mesenchymal stem cells" (BM MSCs). Similarly, adipose tissue can be harvested, processed through enzymatic or mechanical methods, centrifuged, and either applied with or without culture expansion. The non-cultured product is often referred to as stromal vascular fraction (SVF), with yields ranging from 4737 cells/mL of adipose tissue to 1,550,000 cells/mL of tissue [12]. SVF can also

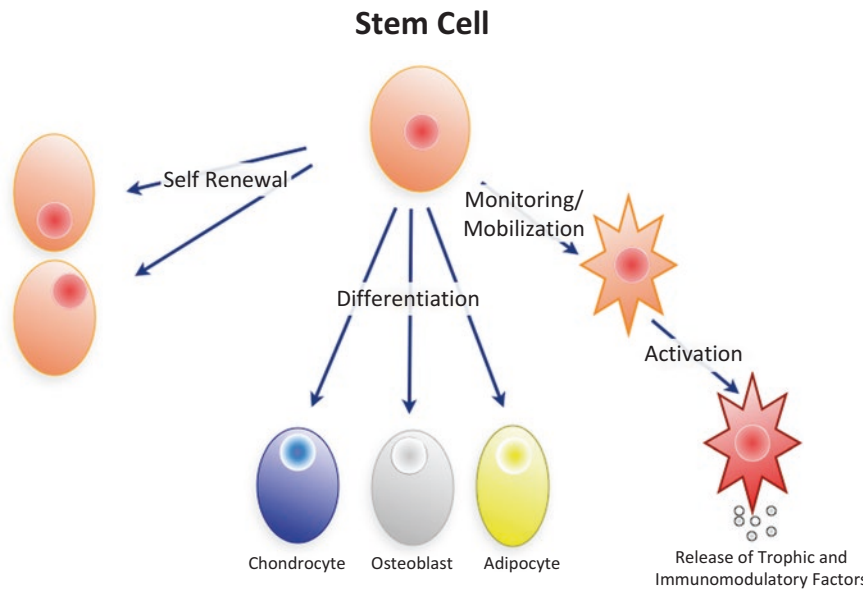

Fig. 13.1 The four capabilities attributed to stem cells: replicate, differentiate, monitor/mobilize, and exert paracrine effects. (Reprinted from Anz [10]. With permission from Springer Verlag)

be cultured to increase stem cell yield, and the product is called adipose-derived stem cells (ADSCs). One must consider all steps involved in a product's preparation in order to evaluate its potential value. Developmental hurdles of culture expansion include risks involving bacterial contamination and cellular transformation. Within the United States, the FDA has ruled that they consider cultured cells as drugs which require pre-market development and approval [13].

Preclinical In Vitro Development

Alexander Maximow is a Russian-born scientist who is credited with the earliest discoveries involving stem cells. His work at the University of Chicago in the 1920s included "morphology of the mesenchymal reactions" and the development of cells into fibroblasts in vitro [14, 15]. Further work on cells from bone marrow progressed in the 1960s [16], but the foundational work applying stem cells to cartilage repair started in the lab of Arnold Caplan in the late 1970s [17, 18]. Caplan and his colleagues were the first to differentiate cultured cells from bone marrow aspirate into multiple tissues including chondrocytes, adipocytes, and osteocytes. Initially, studies began with inducing embryonic chick limb mesenchymal cells to differentiate into cartilage cells. Since the end-stage cells arose from mesoderm, the term mesenchymal stem cell (MSC) was coined [19]. Caplan's work progressed and tracked to orthopedic applications leading to the release of his monograph entitled "Mesenchymal Stem Cells" in the *Journal of Orthopedic Research* in 1991. In this paper, he proposed that 1 day MSCs could be isolated from autologous tissue, culture expanded ex vivo, and reimplanted for differentiation into repair tissue, such as cartilage or bone [7].

Scientists all over the world have continued to study stem cells from diverse sources uncovering mechanisms of cell differentiation and cell signaling. To review all in vitro studies is beyond the scope of this article; important lessons in the process will be highlighted. It has become clear that stem cells can be induced into cartilage cells, with work starting with bone marrow-derived cultured cells [20]. Additionally, cells from other tissue sources have shown potential to differentiate to cartilage including cells derived from fat, periosteum, synovium, and muscle [20–24]. Initial comparative studies proved that bone marrow-derived cells have more chondrogenic potential than adipose-derived cells [25, 26]. Further differentiation studies compared cells derived from bone marrow, synovium, periosteum, fat, and muscle with superior cartilage growth from cells derived from bone marrow and synovium [23] (Fig. 13.2). Later direct comparison of synovium to bone marrow determined that synovial-derived cells have the greatest chondrogenic potential [23, 27]. Recent studies have identified stem cells in different layers of cartilage with emerging mechanisms in cartilage maintenance and repair response [28–32]. Early in vitro and in vivo studies are investigating the potential of these stem cells compared to other mesenchymal sources [24, 33]. Considering multiple cell sources have proven productive in benchtop study, the logistics around processing and application in light of regulatory/developmental requirements will likely guide clinical applications.

Preclinical Animal Development

Similar to benchtop review, the entirety of animal study is beyond the scope of this article, and highlights will be made. Benchtop work was first translated to animal experiments in the early 1990s. Building upon Caplan's work and with his collaboration, Wakitani et al. [34] implanted bone marrow-cultured MSCs on a collagen gel into a cartilage defect in rabbit model. The MSCs differentiated into chondrocytes by the 2nd week after implantation, and by the 24th week, tissue had organized into cartilage tissue and a subchondral bone plate redeveloped (Fig. 13.3). This study provided proof of Caplan's concept that cells could be harvested, cultured ex vivo, and reimplanted for tissue repair. Similar studies have been performed with adipose tissue [35, 36], synovium [37, 38], and periosteum [39]. In a

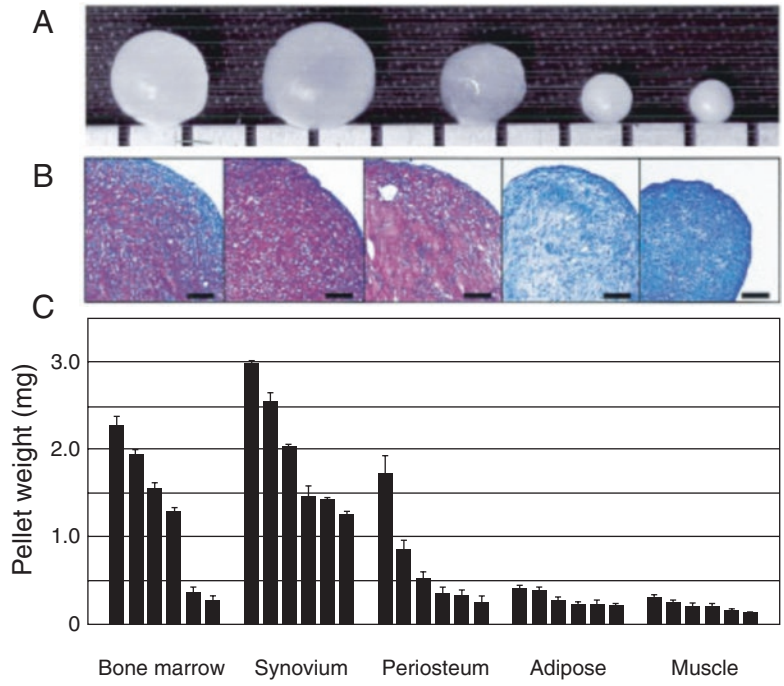

Fig. 13.2 Chondrogenic potential of 5 human tissue sources after 3 culture passages followed by pelleting the cells and incubating for 21 days. Panel (**a**) represents the gross appearance next to a 1-mm scaled ruler. Panel (**b**) represents the histologic appearance with toluidine blue staining. Panel (**c**) compares the wet weight of the pellets from 6 individual donors. Values are the mean and SD of 3 samples from each source in each donor. (Reprinted from Sakaguchi et al. [23]. With permission from John Wiley & Sons)

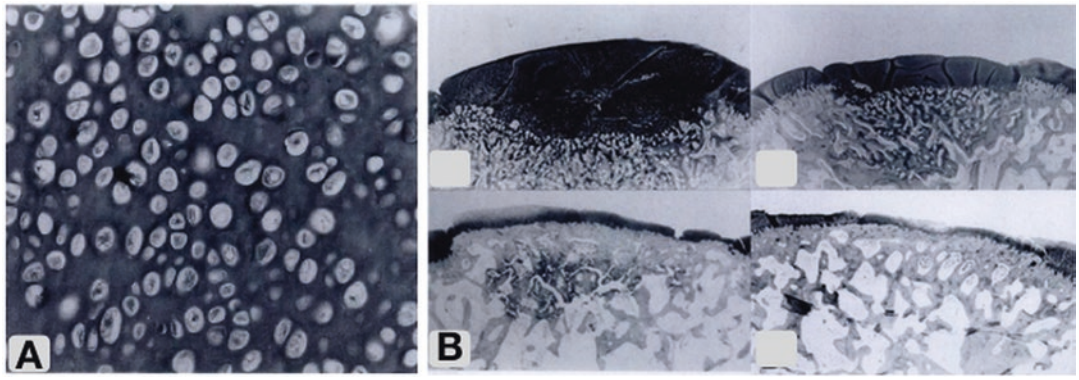

Fig. 13.3 In a rabbit model BM MSC implanted into a cartilage defect in a collagen gel differentiated into chondrocytes by the second week after implantation (**a**) and by the 24th week tissue had organized into cartilage tissue and a subchondral bone plate redeveloped (**b**). (Reprinted from Wakitani et al. [34]. With permission from Wolters Kluwer Health Inc.)

comparative study, bone marrow-derived stem cells were found to have superior cartilage formation ability than those cells derived from adipose cells in a canine model [40].

In addition to implantation of cells within a scaffold, another tested concept is that stem cells injected into a local environment, i.e., a joint, have the potential to home (or localize) to an area of injury and participate in cartilage healing. Lee et al. investigated this concept in a mini-pig [41]. After the creation of a cartilage defect, one group received an intra-articular injection of BM MSC (average seven million cells) suspended in hyaluronic acid (HA) followed by two additional weekly HA injections, another group received three weekly HA injections, and a third group received three weekly saline injections. While both the HA and MSC groups were superior to

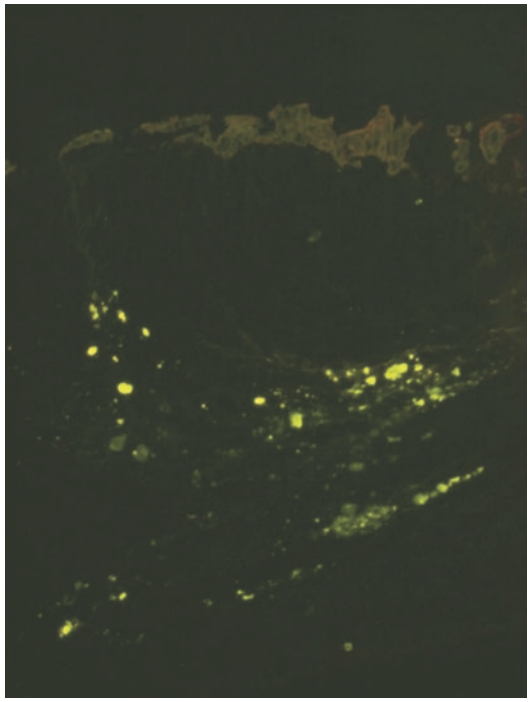

Fig. 13.4 Carboxyfluorescein-labeled BM MSC administered by intra-articular injection after the creation of a cartilage defect in a mini-pig model homed to and integrated into repair tissue. (Reprinted from Lee et al. [41]. With permission from John Wiley & Sons)

saline, the MSC group showed improved histologic and morphologic evaluation. The BM MSC were labeled with carboxyfluorescein, and upon histologic examination, the labeled cells were homed to and integrated into the repair tissue (Fig. 13.4). A similar study of an injection of stem cells instead of direct implantation has been performed with the same conclusions drawn in a meniscus injury model [42], cultured synovial-derived stem cells (SDSC), and a large-animal model and BM MSC [43].

A concept without conclusion is whether immature stem cells or stem cells differentiated toward the chondrocyte lineage perform best in cartilage repair models. In a porcine model, cultured BM MSC embedded in a collagen scaffold were compared to cultured BM MSC pretreated with transforming growth factor β to differentiate the cells toward a chondrocyte line. The repair tissue in the undifferentiated group illustrated superior histologic and morphologic repair tissue. In contrast, in an ovine model, researchers determined the optimal predifferentiation period of MSC with chondrogenic medium in vitro. The predifferentiated cells were then implanted in a hydrogel and compared to undifferentiated MSC in a hydrogel. The predifferentiated cells showed better histologic scores with morphologic and immunohistochemical properties of hyaline cartilage [44].

With consideration of developmental and regulatory hurdles, researchers have also studied bone marrow aspirate concentrate as an adjunct to cartilage repair procedures. The use of bone marrow aspirate concentrate implanted at the time of a marrow stimulation procedure and as a single or series of injections after a marrow stimulation procedure has been shown to improve cartilage repair in an equine and caprine model [45, 46].

Clinical Development Overview

The application of stem cells in human studies has emerged and continues to emerge in three phases: case report/series design, comparative treatment study, and randomized controlled study. A recent systematic review in 2016 found 60 clinical studies including 9 case reports, 31 case series, 13 comparative trials, and 7 randomized controlled studies. On review, 20 of the studies investigated BM MSC, 16 investigated SVF, 16 investigated BMC, 5 studies investigated peripheral blood stem cells (PBSC), 1 study investigated ADSC, 1 study investigated SDSC, and 1 study compared BMC to PBSC. Twenty-six of the sixty studies involved injection of cells intra-articularly for administration, and 33 investigated surgical implantation either in an open or arthroscopic fashion [47]. Generally speaking, stem cell treatments for cartilage repair have been safe and effective yet require further well-designed comparative study. Progress for each cell source will be summarized and landmark studies discussed in depth.

Clinical Development of BM MSC

BM MSC have the longest track record with initial work arising out of Japan. In 2002, Wakitani published a comparative study involving 12 patients undergoing HTO with BM MSC surgically implanted with a scaffold compared to 12 patients who underwent HTO alone [48]. At 16 months, clinical outcomes between the two groups were similar, while histologic and arthroscopic examination revealed better tissue in the MSC group. Subsequent case reports and case series of open surgical implantations with varying surgical methods followed with encouraging histologic and clinical outcome scores [49–55]. Additionally, case series have followed, investigating the injection of BM MSC for the symptomatic treatment of osteoarthritis with encouraging early results [56–61].

Comparative work of note has emerged from a group of research clinicians in Singapore since 2010 [62, 63]. Building on the mini-pig work described in the preclinical animal section, the group reported a comparative study of BM MSC implanted under a periosteal patch versus cultured chondrocytes under a periosteal patch, i.e., autologous chondrocyte implantation (ACI) [62]. In a matched population, there was no difference in clinical outcomes between the groups at 24 months. Intra-cohort analysis revealed that older patients with ACI did not perform as well as younger patients with ACI, while older patients with the BM MSC performed as well as younger patients with BM MSC. Authors concluded that the stem cell method was the less aggressive, less expensive, and less morbid of the two procedures. The initial comparative study was followed by a second comparative study evaluating open periosteal implantation with intra-articular injection of BM MSCs after arthroscopic marrow stimulation [63]. At 24 months there was similar improvement in both groups leading the authors to suggest that the injection method was superior due to less morbidity. In 2013, the group reported on the results of a randomized controlled trial evaluating patients with unicompartmental OA and varus malalignment. Half of the patients were randomized to high tibial osteotomy (HTO), arthroscopic microfracture, and one postoperative injection of HA. The other half of the patients were randomized to HTO, arthroscopic microfracture, and one postoperative injection of BM MSC suspended in HA. At 2-year follow-up, both groups illustrated improved yet similar outcome scores, while the BM MSC group produced better MRI scores [64].

Clinical Development of SVF and ADSC

To date developmental studies of adipose-derived tissue have involved SVF with the exception of one study involving ADSC. The majority of the work has emerged from a group out of South Korea investigating the use of SVF to augment arthroscopic procedures and osteotomy as well as investigating its role for osteoarthritis. Studies started with harvesting adipose tissue from the infrapatellar fat pad and progressed to liposuction harvest from the buttock region. The methodology for the group involves processing the tissue with centrifugation and collagenase and reliably produces four million ADSCs from 120 mL of lipoaspirate. The group has investigated one administration time point via intra-articular injection, arthroscopic implantation without a scaffold with PRP, and arthroscopic implantation with a fibrin scaffold. They conclude that arthroscopic implantation with a fibrin scaffold is safe and the most effective method for SVF administration. They have shown that it can improve the clinical results of simple arthroscopic debridement, marrow stimulation, and osteotomy. Comparison of this technique to other cartilage repair procedures is lacking at this time. This group has reported significant clinical and morphologic improvement when evaluated with MRI, yet histologic results have shown room for further development. These authors have determined that older age, higher BMI, and a larger defect size were negative predictors in all studies [65–74].

One study has investigated the dose-response relationship of ADSC to treat degenerative cartilage lesions with injection. The first phase of the

study compared a 10 million cell injection, a 50 million cell injection, and a 100 million cell injection, with the best results reported in the 100 million injection group. The second phase followed nine patients receiving a single 100 million cell injection. No treatment-related adverse events were reported. WOMAC scores remained improved at 6 months after injection in the high-dose group. Second-look arthroscopy and histology suggest the regeneration of cartilage in the high-dose group [75].

Clinical Development of PBSC

Clinical results have also been emerging with PBSC. This concept follows the footsteps of the hematology oncology profession with development of the harvest of stem cells for bone marrow transplant. While originally bone marrow transplant involved bone marrow aspiration harvest, the profession developed harvest via pharmaceutical mobilization and venous harvest with apheresis. Pharmaceutical mobilization stimulates an upregulation of production of stem cells in the bone marrow and release of these cells to the peripheral circulation. Apheresis harvest involves a machine which uses centrifugation, optics, and continuous venous access for a period of 1–4 h to collect PBSC. For example, with orthopedic indications in mind, a 140-mL harvest contains on average 140 million PBSC, and the harvest can be aliquoted and stored for serial/multiple injections [76] (Fig. 13.5). This cell source has established safety data involving large registries and characterization of the cells, suggesting that they are more immature than BM MSC and have functional properties similar to ESC [1, 77]. One striking advantage of this cell source is the ability to harvest at one time point millions of cells which can be aliquoted and stored for serial injections throughout the

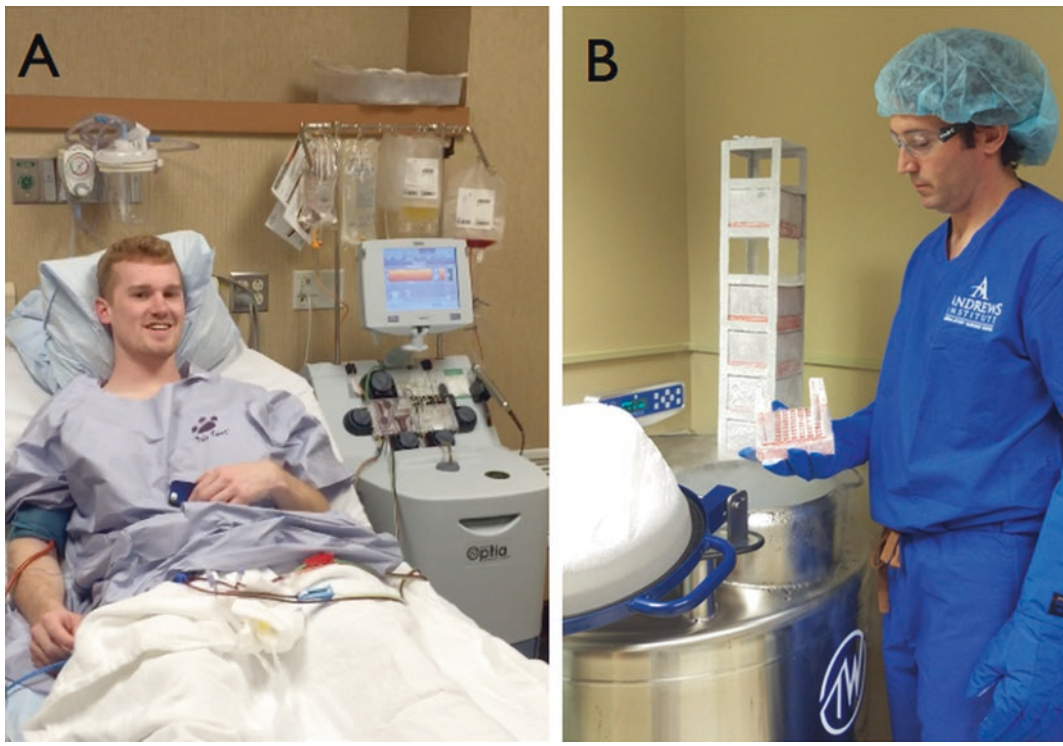

Fig. 13.5 Pharmaceutical mobilization of peripheral blood stem cells to the blood and closed-loop apheresis harvest (**a**) over 1–4 h allow for the harvest of millions of stem cells which can be aliquoted and stored (**b**) for serial injections. (Reprinted from Anz [10]. With permission from Springer Verlag)

maturation phase of the cartilage healing. This method leverages the body's potential to create stem cells and does not require cell culture to produce hundreds of millions of cells.

The majority of developmental work applying PBSC to cartilage repair has emerged from a group in Malaysia. Saw et al. first reported a case series involving arthroscopic marrow stimulation followed by multiple postoperative intra-articular injections in five patients, with safety data and histology suggesting good cartilage repair tissue [76]. The case series was followed by a RCT comparing arthroscopic marrow stimulation followed by 8 postoperative PBSC intra-articular injections over the course of 6 months compared to arthroscopic marrow stimulation followed by 8 postoperative HA intra-articular injections. At 2 years, histology and MRI results favored the treatment group, but the clinical outcomes scores did not reveal superiority. On average, each stem cell injection in the intervention group contained eight million stem cells [78]. This group recently published a case series combining the cartilage procedure with HTO. Repair cartilage in this combination procedure when graded with ICRS scoring system approached 95% of that of normal articular cartilage. Similar encouraging results were seen in two additional case series involving PBSC and one comparative study of open implantation of PBSC to BMC [79–81].

Clinical Development of SDSC

Research involving the use of synovial-derived cultured cells has been arising from Japan. In one study, synovial-derived cultured cells have performed well at 3 years in a case series of 10 patients with single cartilage defects with a median size of 2 cm^2, illustrating improvement in a MRI score, qualitative histology, and outcome scores. Administration involved culture expansion for 14 days, followed by arthroscopic application, allowing the suspension to rest in the horizontally placed defect for 10 min to allow adherence of the cells [82] (Fig. 13.6). Alternative work has developed a scaffold-free tissue-engineered construct from SDSC. A safety trial involving 10 patients was completed in 2015 with encouraging 1-year results [83].

Clinical Development of BMC

With regulatory hurdles to the clinical implementation of cultured cells in all modern countries, clinical researchers in Italy have pioneered the direct surgical implantation of bone marrow concentrate involving a hyaluronic acid matrix. In 2009, a prospective clinical study reported on the repair of talar osteochondral lesions in 48 patients. With a minimum follow-up of 24 months, clinical results improved. Histology results illustrated variable tissue quality, with none being entirely hyaline cartilage [84]. Work continued with multiple case series involving the knee and ankle and culminated with a randomized controlled trial in the ankle and a prospective comparative study in the knee [85–97]. Of these, a prospective knee study evaluated 37 patients with large patellofemoral chondral defects and compared BMC under a HA scaffold method to matrix-induced autologous chondrocyte implantation (MACI). Both groups showed significant improvement in clinical scores, and there was no significant difference in improvement between the two groups, except for the IKDC subjective score, which favored the BMAC group [97]. Subtle superiority was observed in the BMC group including deterioration in MACI from 2-year to final follow-up and anatomic defect location proving a hurdle for the MACI group. Upon MRI review, complete filing was observed in 76% of patients in MACI and 81% of patients in BMAC. Biopsies were obtained in four patients in each group with analysis revealing hyaline-like features (Fig. 13.7). A similar comparative study was performed in 80 patients with osteochondral lesions of the talus. Clinical results were similar in both groups at 48 months, with subtle superiority of the BMC group in return to sport and MRI evaluation [94].

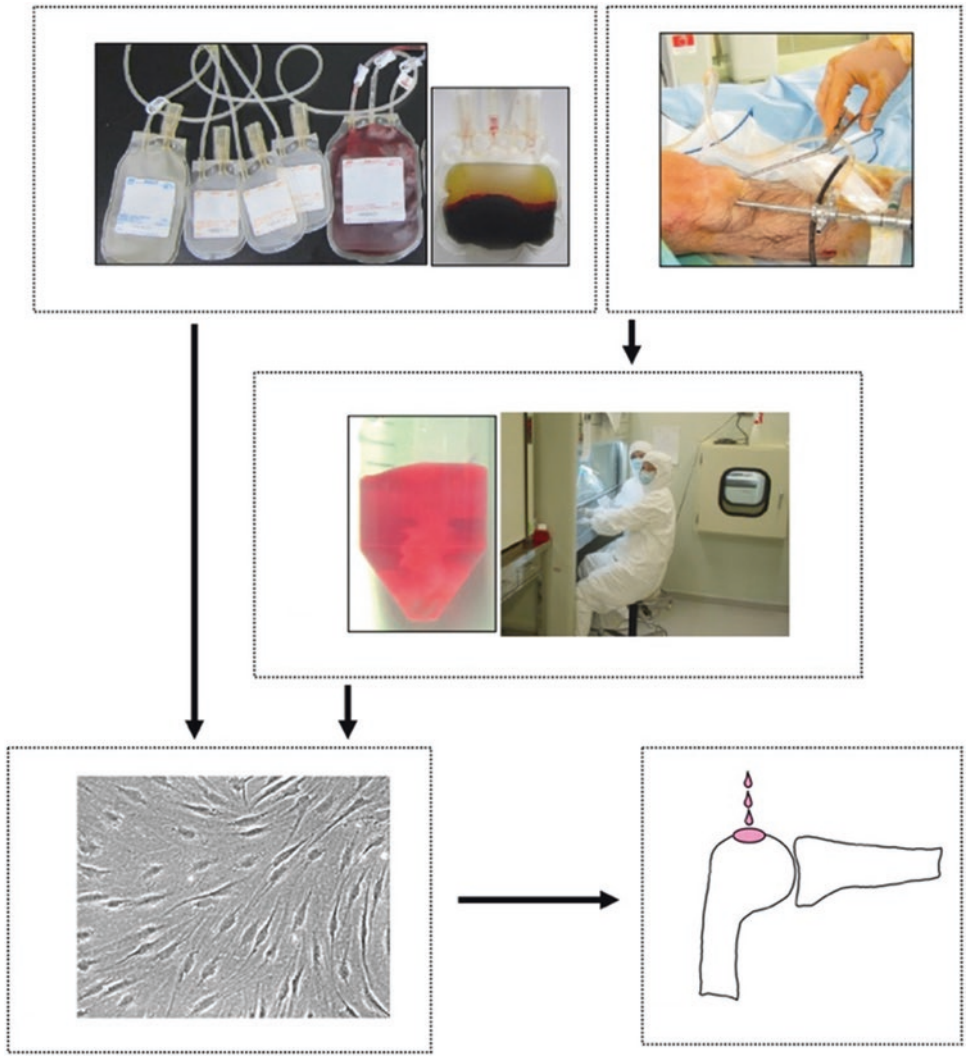

Fig. 13.6 Synovial-derived cultured cells have performed well at 3 years in a case series. Methods involved arthroscopic harvest culture expansion for 14 days in a clean room and an arthroscopic administration which involved holding the defect upward and allowing the suspension to sit for 10 min to allow adherence of the cells. (Reprinted from Sekiya et al. [82]. With permission from Creative Commons Attribution 4.0 International License: http://creativecommons.org/licenses/by/4.0/)

Conclusion

The application of stem cells to cartilage injury has come a long way. The translation from bench to bedside has taken 40 years, but clinical success has been documented in several trials. Varying approaches are being developed around the world owing to different and evolving regulatory requirements. It appears that with high cell numbers and repeated administration, intra-articular injection is viable, while one-step surgical implantation is preferred for low cell number and single-point administration technologies, i.e., cell concentrate technologies. We look forward to the coming decade of clinical development and postulate that within that decade we will see multiple technologies available for patient care.

Biopsy Specimens Reports.

		Histopathology[a]					
Cases		Surface	Structure	Proteoglycans	Cells	Subchondral Bone	Immunohistochemistry
MACI	1	Smooth	Not well organized	Not represented	Not represented	Active remodeling	Col 1: E/C, Col 2: I/C
	3	Smooth	Well organized and slightly fibrous	Represented	Columnar disorganized distribution	Active remodeling	Col 1: few positive cells, Col 2: E/C
	4	Smooth	Well organized	Represented	Columnar homogenous distribution	Active remodeling	Col 1: absent, Col 2: E/C
	5	Smooth	Well organized	Represented	Columnar homogenous distribution	Active remodeling	Col 1: absent, Col 2: E/C
BMAC	1	Smooth	Well organized	Represented	Columnar homogenous distribution	Active remodeling	Col 1: absent, Col 2: E/C
	2	Smooth	Well organized	Represented	Columnar homogenous distribution	Active remodeling	Col 1: absent, Col 2: E/C
	4	Smooth	Not well organized	Not represented	Not represented	Active remodeling	Col 1: E/C, Col 2: I/C
	5	Smooth	Well organized and slightly fibrous	Represented	Columnar homogenous distribution	Active remodeling	Col 1: absent, Col 2: E/C

MACI = matrix-induced autologous chondrocyte implantation; BMAC = bone marrow aspirate concentrate; Col = collagen type; E/C = extracellular; I/C = intracellular.

[a]Hematoxylin and eosin or safranin-O staining.

Fig. 13.7 Histologic comparison of matrix-assisted chondrocyte implantation to bone marrow aspirate concentrate implanted in a HA scaffold showed hyaline-like features in 75% of the specimens in both groups. (Reprinted from Gobbi et al. [97]. With permission from SAGE Publications Inc.)

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