Chapter 14
The Role of Bone Marrow-Derived Mesenchymal Stem Cells in Sports Injuries

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14.1 Introduction

Bone marrow-derived mesenchymal stem cells (BM-MSCs) have been found to play an important role in tissue repair after injuries of the musculoskeletal system. Fat, cartilage and bone can derive from their differentiation (Pittenger et al. 1999; Charbord et al. 2010; Kagami et al. 2011).

Musculoskeletal injuries commonly occur during sports. Three percent of patients visiting Scandinavian hospitals as emergencies have injuries related to sports, and every year 22.5 out of 1000 citizens will experience such an injury (de Loes 1990; Lindqvist et al. 1996). These figures highlight the significance of this type of injuries. Innovative and more efficient treatment modalities are therefore under research, and BM-MSCs could be one of them. It is important that the results and findings of this field of research are reviewed with aim to reach clear and rigid conclusions.

Bone, cartilage, ligament, tendon, muscle and meniscus are the tissues that are usually affected during sports injuries. Their treatment with the use of BM-MSCs is the subject of this review.

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14.2 Muscle

Abnormal loading of muscle can occur during sports. Therefore, injuries varying from strain to muscle tears can occur, and the mobility of the patient can be impaired. Strains of the hamstrings had an incidence of 86.4 for every 10,000 player hours in three seasons in Australian football (Orchard et al. 1998).

Resident BM-MSCs are the primary mediators of muscle repair and remodelling. These multipotent cells migrate into the regenerating muscle and form a cell population with potential to differentiate to muscle. In more severe injuries the population of muscle precursors in a more advanced stage of differentiation is maintained by BM-MSCs. However, their effect on stem cells when given systematically is minimal, and their use in the treatment of sport-related injuries is proven ineffective (Ferrari et al. 1998). On the other hand, human adipose tissue (AT)-derived MSCs were more efficient in generating muscle than MSCs from bone marrow (BM) and synovial membrane (SM), as shown in a recent study from three donors. Their myogenic potential, along with the nondemanding harvesting, renders AT-MSCs the optimal source of multipotent cells (de la Garza-Rodea et al. 2011).

Muscle repair by BM-MSCs in adults takes place as a staged process, as described by LaBarge and Blau (2002). Two different biological factors stimulate a BM-MSC response. BM-MSCs have the capability to occupy the stem cell niche in muscle following damage induced by irradiation that caused ablation of resident cells. Furthermore, after damage generated by exercise, multinucleated muscle fibres showed regeneration in which BM-MSCs played a role at a much greater frequency than any other reported for conversion of bone marrow to muscle. This suggests that BM-MSCs could serve as cell supply for damaged muscle repair.

14.3 Tendon

Some tendons serve a binary role of transmitting force from muscles and storing elastic energy (Fukashiro et al. 1995). A good example is the Achilles tendon which is crucial in long-distance running and suffers fatigue injury and rupture after prolonged cyclical loading (Kvist 1994). In a study of partial tendon ruptures (Kvist 1994), three quarters of the injuries were caused during sports activities involving sprinting and jumping repeatedly in a 14.9 odds ratio compared to controls. On the other hand, tendinopathy of the Achilles tendon is more common in endurance runners with an equivalent ratio of 31.2 (Kujala et al. 2005). Tendinopathy of the patella tendon is another injury of the same category, potentially career ending, which can significantly impair function (Koen and Roeland 2005).

The effect of a BM-MSC-seeded fibrin gel in a rabbit Achilles tendon has been studied (Chong et al. 2007). Compared to the plain fibrin gel, after 3 weeks, the modulus of elasticity was 32 % higher, and the percentage of type I collagen fibres increased. However, there was no difference between groups at 6 and 12 weeks.

In another study of Achilles tendon injuries in rabbits, the treatment consisted of either a PLGA scaffold seeded with BM-MSCs (group 1) or a plain scaffold (group 2). The control group (group 3) had no induced injury. Formation of new tissue and remodelling were greater in group 1. At 12 weeks the histological samples were similar to uninjured tendon in both groups. In group 1 the modulus of elasticity was 62.6 % and the tensile stiffness 87 % compared to native tendon. In group 2 both values were lower (52.9 and 56.4, respectively). In conclusion, there appears to be a prolonged effect (Ouyang et al. 2003).

A composite consisting of collagen gel and BM-MSCs was used in lacerations of patellar tendons in rabbits. The outcomes of the study showed better biomechanics such as a 15 % increase in stiffness compared to controls with acellular gel. Well-structured collagen, closer to normal, was found in 40 % of samples. The other 60 % were no different to the gel alone receiving rabbits. The outcome of this study implies that with the use of BM-MSCs, there is improvement in biomechanics, but not in histology of the healed tendon (Awad et al. 1999).

Injury at the bone-tendon junction was studied as well (Ouyang et al. 2004) with BM-MSCs seeded in fibrin glue. Cell-free fibrin glue in the contralateral leg was used as control. In the samples that did not receive BM-MSCs, fibrovascular tissue could be seen in the interface, and at 6 weeks type I and III collagen fibres in a perpendicular orientation, resembling Sharpey's fibres, were visible. The fibrin glue/BM-MSC group had more extensive growth of similarly oriented type I, II and III collagen fibres. This highlights the potential towards formation of tissue similar to fibrocartilage that improves healing.

Furthermore, Gulotta et al. (2010) provided evidence that additional modifications to MSCs can be performed to improve tendon healing. He used 60 rat models with induced detachment of the supraspinatus tendon of one side. MSCs seeded in fibrin glue were administered to half of the rats, and adenoviral MT1-MMP (Ad-MT1-MMP)-transduced MSCs to the other half. The second group had statistically significant higher stiffness values, more fibrocartilage, higher ultimate stress to failure and higher ultimate load to failure.

The mechanical properties of MSC-treated tendons subjected to cyclic loading may be improved as well. Acellular paw flexor tendons in rabbits were divided in three groups: normal tendons, tendons reseeded with MSCs and tendons reseeded with fibroblasts (Angelidis et al. 2010). Fifty percent were subjected to cycle loading and 50 % were immobilised for 5 days. The tendons under loading showed significantly higher modulus of elasticity and ultimate tensile stress compared to all the incubated tendons and similar values to the fresh tendons that underwent cyclical loading.

The above studies showed that with the use of BM-MSCs the biomechanical properties of tendons tend to improve. It could be just because cells create a larger cross-sectional area, but the histology of the samples does not confirm the results. These results are of low significance when compared to normal tendons. Awad et al. (1999) showed that the strain energy, maximum stress and modulus of elasticity were respectively 32 %, 16 % and 7 % of healthy patella tendons. As highlighted in the studies, with the use of BM-MSCs, there is improvement in the healing process of the tendons, but the effect on the outcome varies.

14.4 Ligament

During vigorous movement in sports, joint stability and normal tracking are achieved with the function of ligaments. Furthermore, the proprioceptors that they contain provide input to the central nervous system. This is how complex motion patterns are achieved in sports (Frank 2004).

Anterior cruciate and medial collateral ligaments (ACL and MCL, respectively) are commonly injured in athletes. The mechanism of an ACL injury could be either a direct valgus force to the knee or torsional stress with the foot firm on the ground or during landing. The ACL injury incidence in the population of Denmark is 3/10,000 annually, more common in athletes (Nielsen and Yde 1991). They are the most frequent injuries in Sweden in some sports, reaching 43 % in soccer. They are potentially career-changing injuries, as 3 years post injury only 30 % of soccer athletes continue playing (Roos et al. 1995). Apart from their career-ending severity, injuries in ligaments increase implications such as osteoarthritis in the future (Gillquist and Messner 1999).

Successful treatment is crucial to athletes for their adequate recovery and return to sport. Operative treatment is a reasonable choice in partial or complete tears. It can be performed either by reconstruction with the utilisation of intra- or extraarticular tendon autografts and the expected morbidity of the donor site or by direct repair with sutures (Gobbi et al. 2009). BM-MSCs could potentially be used as the main treatment (Kanaya et al. 2007) or as an adjunct to other treatments currently in use (Lim et al. 2004). BM-MSCs have shown greater production of collagen and proliferation compared to skin and ACL fibroblasts. On the downside, these conclusions have been reached after testing on one animal only (Van Eijk et al. 2004).

ACLs with partial rupture in 98 rats were used to study the effect of injection of BM-MSCs directly in the knee (Kanaya et al. 2007). The ACLs on the right side were partially torn and the left side was subjected to a sham procedure. A phosphate-buffered solution (PBS) containing autologous BM-MSCs was injected in one group, whereas the control group received the same amount of pure solution. The femur/ACL/tibia complex in the first group showed a significantly increased ultimate load to failure (70.5 %) compared to the controls (58.2 %). The area of transection in the PBS ACLs was retracted, and no histological signs of healing were seen at any post-operative points. On the other hand, no retraction occurred in the group that received BM-MSCs, and BM-MSC-containing reparative tissue bridging the rupture was present from 14 days post-operatively. The histological score that was used showed much better grades for the BM-MSC group over the PBS group. The stem cells survived the environment of the knee joint and promoted the repair of the partially ruptured ligaments leading to better biomechanical and histological results.

One of the downsides of reconstruction of ligaments is the pullout of the tendinous autograft in the early post-operative period. The effect of BM-MSCs in the enhancement of the reconstruction has been studied. Reconstructions of ACLs were performed bilaterally in rabbits using hamstrings as tendinous autografts. The autograft of the limb under treatment received an autologous BM-MSC-seeded fibrin

glue coating. Plain fibrin glue was used in the control limb. At 8 weeks post-operatively, the treatment group had zones of matured cartilage with high concentration of type II collagen, similar to entheses of normal tendons. In the control group however, reparative tissue rich in type I and III collagen was found in the bone-tendon junction, with fibres similar to Sharpey's. Overall, the BM-MSC group had significantly higher mean load to failure and stiffness (66 % and 51 %, respectively) in comparison with the control group. However, 44 % of the BM-MSC group reconstructions underwent pullout when tested, leaving this problem unsolved. Nevertheless, the use of stem cells led to stronger ligament reconstructions with closer to normal biomechanical properties.

Clear evidence has been presented in favour of BM-MSCs as cell source for ligament reconstruction, as they improve their histological, physiological and biomechanical properties. BM-MSCs have been applied as treatment in various ways with equivalent results that highlight their potential.

14.5 Bone

High-demand sports like running submit bone to loading cycles of high impact. The multiple microfractures that are caused this way can progress to larger size splits. Stress fractures have an incidence of 21.1 % in athletes annually and represent 20 % of the injuries of the musculoskeletal system. Jumps, sprints and hurdles are frequently related to foot fractures. Pelvic and long-bone fractures are associated with long- and middle-distance running (Bennell et al. 1996).

The average duration of the healing process in closed fractures of the tibia is 28 weeks (Wiss and Stetson 1995). Fast treatment with good results is mandatory for athletes, as they stay away from training during that period. The quality and speed of fracture healing with the potential aid of BM-MSCs has been studied.

A comparative study was performed in sheep between coral scaffolds seeded with or without BM-MSCs and scaffolds enriched with fresh bone marrow (FBM) or bone defects without treatment. Degradation of the scaffold occurred at an equal rate with the deposition of bone in the BM-MSC scaffold. Only with this scaffold did union resemble normal bone. It was achieved 16 weeks after the initiation of treatment. On the other hand, degeneration was faster than deposition in FBM and coral scaffolds. However, the latter showed osteoconductive properties as new bone had formed in the centre of the medullary canal (Petite et al. 2000).

In another study assessing the BM-MSC effect in rat femoral fracture rates of healing, a ceramic cylinder consisting of a mixture of tricalcium phosphate and porous hydroxyapatite was used. In one side the cylinder was used in its simple form. The other leg received a BM-MSC-seeded cylinder. Empty defects in some animals were used as controls, and non-union was the outcome in all of them. The BM-MSC side was united in 12 weeks with bone growing in the scaffold pores. In comparison to the scaffold-only group, increased values of absorption of torsional energy (212 %), stiffness (245 %) and strength (215 %) were documented. In the

plain scaffold limb, the implant pores were filled mainly with connective tissue (Bruder et al. 1998b).

In a different study on fracture healing in dogs, a 65 % hydroxyapatite and 35 % β-tricalcium phosphate ceramic cylinder scaffold was used. The first group received just the scaffold and the second a BM-MSC-enriched cylinder and the third was left untreated. No callus was seen and no bone was formed in any of the animals of the last group, where all the fractures resulted in non-union. In the cylinder-alone group, fibrous tissue filled the implant pores, visible callus formation was not present and cortex-implant union was achieved in ten out of twelve dogs at 16 weeks. In the stem cell group, there was even distribution of bone around and inside the cylinder, and good integration with native bone was noted. Satisfactory union was achieved in 12/12 animals in the group at 8 weeks, with continuous bone formation with good mineralization around the defect. In 84 % of animals, there was good osseous callus formation surrounding the implant (Bruder et al. 1998a).

Tibial fractures in mice treated with BM-MSC insertion compared to a cell-free control group showed formation of callus of improved strength and reduced stiffness and better final displacement rates. As a result the rigidity was decreased and the callus was less brittle. The new bone was increased in quantity and better mineralised, and the callus was larger, all due to BM-MSCs. The bone properties and the process of fracture healing were improved because of the formation of a better structured bony bridge (Granero-Molto et al. 2009).

A hydrogel scaffold based on gelatine was documented to support differentiation towards bone of BM-MSCs (Ben-David et al. 2011). Osteogenic differentiation was noted in mesenchymal stem cells that were cultured in an osteogenic environment on hydrogel. This was proven with the formation of Alizarin Red staining-positive cluster, mRNA osteogenic marker expression and calcium phosphate sedimentation confirmed with spectroscopy and scanning electron microscopy,

Currently, bone autografts are the treatment of choice for sizeable bone defects. Nevertheless, there is a limit to the available quantity, and the donor site morbidity is an issue, including functional impairment, infection and nerve injury. Scaffolds seeded with BM-MSCs are a different option providing more safety and less morbidity, and there is evidence that deposition of new bone, biomechanical properties of the callus and duration of the healing process are all satisfactory.

14.6 Meniscus

The main function of the menisci is to disperse loads over a larger surface, reducing the stress to the cartilage in the joints and thus decreasing the osteoarthritis risk. They also participate in articular lubrication and during loading they play a shock-absorbing role. Their structural properties render them efficient in resisting stress in rotation, translation and compression (Walsh et al. 1999). In ACL- or PCL-deficient knees, stability is provided by the menisci (Fithian et al. 1990).

An ACL injury has an accompanying meniscal tear in 62 % of cases (Noyes et al. 1980). In contact sports, the combination of ACL, MCL and medial meniscal tears forms the O'Donoghue triad (O'Donaghue 1950). Injuries in the red (vascular) zone of the meniscus have a good healing potential, as opposed to injuries in the white (avascular) zone. Allografts have been used to address that issue with inconsistent results. Cell isolation from the injured region after resection is another option, but with poor cell quality and quantity. Replacement with prostheses is under investigation with encouraging results in animal studies. BM-MSC scaffolds are a different treatment proposal in current research.

Fibrin glue was used as a scaffold plain or seeded with BM-MSCs in rats. A control group received no treatment and showed no formation of extracellular matrix (ECM) although a large number of polygonal cells were seen in the defect by 12 weeks. Twenty-five percent of samples from the group with the acellular scaffold at 12 weeks contained numerous ECM-producing small round cells inside the fibrin glue. Abundant round cells with ECM around them were found at 12 weeks in 75 % of specimens in the BM-MSC-seeded scaffold group, with visible cartilage-resembling tissue (Izuta et al. 2001).

In a different study, rabbit menisci with circular punch injuries not larger than 2 mm in their avascular area were either left without treatment or treated with an acellular composite collagen-hyaluronic matrix, platelet-rich plasma (PRP), autologous MSCs or bone marrow (Zellner et al. 2010). In other studies, MSC-seeded matrices were cultured in a chondrogenic environment for 2 weeks prior to implantation. Unrestricted movement in cage was allowed to rabbits for 4 months. Healing tissue with fibrous appearance was noted in acellular implants and untreated injuries. Compared to acellular implants, bone marrow and PRP did not improve the healing response. MSCs in a hyaluronic-collagen matrix enhanced the healing response with production of tissue similar to native meniscus inside the avascular areas.

In another study on rabbits, the results of type I collagen cell-containing scaffolds were investigated (Walsh et al. 1999). Partial meniscectomy was performed in all animals bilaterally. One group was left untreated, the second received periosteal autograft, the third type I collagen sponge and the fourth sponge seeded with BM-MSCs. Especially in late stages of treatment, osteoarthritis was noted in all four groups. It was more severe in the autograft group and showed improvement in the sponge groups, mainly the one containing stem cells. The presence of stem cells in the scaffold promoted the production of fibrocartilage, and mature proteoglycan and collagen bundles were seen. There was a smooth transition from healed to physiological meniscus.

Rabbits were subjected to pars intermedia resection and were treated with a hyaluronan/gelatine scaffold or BM-MSCs or left untreated. The specimens that received BM-MSC treatment showed abundance of cells with chondrocyte morphology and ECM similar to normal meniscus, but there was a lower level of order, and the collagen fibrils were smaller in diameter. The defects were filled almost completely and the scaffolds were well integrated. The quantity of fibrocartilage was much higher compared to the plain scaffold. In the empty scaffold specimens, the defects were filled partially and were well integrated with the reparative tissue, but no type II collagen

was found in the ECM produced by the cells. The defects were not filled in the untreated group, and no significant healing process was noted (Angele et al. 2008).

A different study in goats investigated the result of BM-MSC application in a blood clot with sutures in a middle third meniscal defect. Only sutures were done in the first group, a blood clot was added in sutures in the second group, the previous two plus BM-MSCs in the third and no treatment was given to the fourth group. In the first group all eight specimens were healed, four fully and four partially (three at 75 %, one at 75 %). The reparative tissue was not organised, and focal hypercellular areas containing mainly macrophages, leucocytes and giant cells were seen. In the second group there were seven healed specimens, five fully and two partially (50 %), and one repair failed. The healed section was better organised and less cellular compared to the first group. The third group had three healed fully, one partially at 25 % and four failures. The repaired section contained a lower number of cells, and the ECM was increased and with a higher degree of orientation. The last group had seven failures and one partially (25 %) healed specimen. The conclusion of this study highlighted the detrimental effect of BM-MSCs to meniscal tear healing (Port et al. 1996).

All previous studies except the last one found that ECM production by BM-MSCs promotes healing of meniscal injuries. The lack of vascularity in that area does not inhibit their function, making them an optimal treatment option. The different results in the last study could be attributed to design variations such as in vivo insertion of cells into the blood clot as opposed to culturing within the matrix before insertion leading to better BM-MSC retention.

14.7 Cartilage

Articular surfaces are resistant to wear and friction is minimal due to the presence of cartilage. Their capacity to bear loading and absorb shock is high when, during sport activity, intense mechanical forces are applied in joints (Williams et al. 2007). Sporting activity is the cause of 49 % of lesions in articular cartilage (Aroen et al. 2004). The cartilage healing potential after damage is low, and small defects may lead to gradual degeneration and subsequently osteoarthritis (Redman et al. 2005). Treatment that can promote healing is therefore essential to preserve joint function in the long term.

Treatment methods currently applied such as osteochondral autografts, arthroscopic repair and ACI (autologous chondrocyte implantation) have promising results but have significant disadvantages. The results of BM-MSCs and ACI were compared (Nejadnik et al. 2010). Both methods showed significant improvement clinically with similar success rates. Thus, their effectiveness in cartilage healing is equal clinically, but stem cells have the benefits of lower invasiveness and morbidity of the donor site, one anaesthesia instead of two and reduced cost (Nejadnik et al. 2010). Production of repair tissue is induced by microfractures or abrasion arthroplasty. However, its fibrous nature leads to different morphological and functional

properties (Hunt et al. 2002). Likewise, the size of the defect (Hangody et al. 2003; Jakob et al. 2002) and the morbidity at the donor site (Redman et al. 2005) limit the transfer of osteochondral blocks. Therefore, alternative BM-MSC treatment has been applied in studies in order to overcome such limitations.

BM-MSCs were suspended in Ham's F12 nutrient mixture before insertion into a full-thickness defect of patella groove cartilage in rabbits (Im et al. 2001). The controls had acellular mixture injected. At 14 weeks, repair tissue similar to native cartilage with a restored bone layer subchondrally was seen in the stem cell group. Nevertheless, in the controls the repair tissue was thinner, containing less type II collagen in the matrix, non-differentiated and irregular. Also, the BM-MSC group showed markedly better outcomes than the control group, as shown by higher grades in histological scores (14.8 and 8.9, respectively). However, repair tissue-native cartilage junctions were present and could turn into a degeneration starting point after the initial period of 14 weeks. In addition, cell leakage from the repaired defect was noted due to the technique applied. As a conclusion, BM-MSC application in this manner promotes the repair of the articular cartilage but does not essentially lead to reparative tissue of cartilaginous nature (Im et al. 2001).

A full-thickness defect 3×6 mm in size was created in the load-bearing area of the medial femoral condyle in rabbits (Wakitani et al. 1994). Type I collagen gel containing periosteal or bone marrow stem cells was implanted into the defect. The knee of the other side was used as control with either an empty defect or filled with acellular gel. In the BM-MSC specimens, repair tissue resembling hyaline cartilage was seen, better integrated by 4 weeks. Nevertheless, by 24 weeks, there was a progressive decrease in quality as the cartilage thickness was reduced below the level of that of the native cartilage, although there was full restoration of the subchondral bone. Histological scores for the controls showed a significantly lower quality repair. Reparative tissue from the group that received stem cells demonstrated greater stiffness than the controls but lower than native cartilage. Periosteum- and marrow-derived stem cells showed similar results, but PD-MSCs also led to more irregular articular surface (Wakitani et al. 1994).

The idea of hybrid scaffolds has been presented in literature. BM-MSCs have been seeded in a simple polycaprolactone (PCL) and a PCL-tricalcium phosphate scaffold. The first one was used in the cartilaginous portion and the second in the bone part (Shao et al. 2006). Fibrin glue was used to adhere the scaffold into large rabbit MFC weight-bearing area defects. Acellular scaffold was used in similar defects as control. Six months later, good integration between native bone and reparative tissue was seen in all BM-MSC specimens. Most of them had tissue resembling hyaline cartilage with glycosaminoglycan and type II collagen and stiffness values close to normal. On the other hand, fibrous reparative tissue with little similarity to normal bone or cartilage and lower degree of stiffness was found in the controls. However, cracks or fissures were noted in some of the specimens in the stem cell group at the site of integration, and they lacked the normal zonal organisation (Shao et al. 2006).

In the same way, osteochondral large defect repair has been investigated with the use of poly lactic-co-glycolic acid (PLGA) BM-MSC-seeded scaffolds (Uematsu

et al. 2005) in full-thickness osteochondral defects that were created in the patellofemoral groove in rabbits bilaterally. The control group received acellular scaffolds or no treatment. The conclusions of this study were no different than the previous ones. However, as in the study by Wakitani et al. (1994, 2002), no thinning of the repair cartilage at 3 months was reported. Zonal arrangement signs were noted in the repair tissue, as opposed to the study by Shao et al. (2006). Cell leaking, which is a drawback in insertion by injection, was prevented by the PLGA scaffolds (Im et al. 2001). In addition, orientation and structure of the zone of repair, as well as differentiation of stem cells, was induced by the scaffolds. Nevertheless, unsuccessful scaffold integration leading to failure of the repair was noted in the BM-MSC group. Thus, the procedure could be improved. The parallel use of growth factors locally may lead to successful treatment of such defects. In a larger defect (4 mm), repair is not achievable without the presence of TGF-β1 in the scaffolds.

Unlike current treatments for cartilage injuries, the advantage of BM-MSCs is the potential to treat larger defects, with less invasive methods and with final repair tissue resembling more to normal cartilage. In all studies, regeneration of subchondral bone was achieved, but the quality of the regenerated cartilage varied. The application of scaffolds is a promising alternative for future treatment methods, as it enhances the stem cell positive effects. Nevertheless, these suggested procedures have significant disadvantages that need to be addressed before clinical application.

14.8 Osteoarthritis

Osteoarthritis (OA) is both a cause as well as the result of injury during sport (Buckwalter and Martin 2004). Common injuries of this category like ACL ruptures have an effect on the biomechanics of the medial aspect of the knee, putting the medial meniscus under stress and leading to early OA of the medial compartment (Sherman et al. 1988). Athletes participating in power, endurance and mixed sports are 2.5 times more likely to seek hospital care for OA as inpatients (Kujala et al. 1994). At present there is no evidence for any effective therapy for OA, and BM-MSCs are considered to be a possible treatment method.

In a study by Murphy et al. (2003), autologous precultured BM-MSCs suspended in sodium hyaluronan without a scaffold were injected into the knee joint. The subjects were goats with induced OA due to resected ACLs and excised medial menisci and were divided into a group that received the solution with stem cells and a control group with plain solution. In the controls, the repair tissue was lacking organisation and was adhered to the proximal tibial surface at 20 weeks. Significant fibre formation, reduction of the ECM amount and presence of reactive osteophytes were noted compared to the other limb. On the other hand, the reparative tissue in the BM-MSC group showed arrangement and was not in contact with the femur or the tibia. Tibial entheses of the menisci with normal function were formed in the reparative area of the meniscal ligaments. The BM-MSCs had a positive effect in the

reduction of OA degeneration visually and histologically in comparison to the controls. At 20 weeks however, notable OA changes and failure of ACL healing were present in all specimens.

In MFC defects in 24 patients with unicompartmental medial OA that were being treated with high tibial osteotomy (HTO), collagen gel with (treatment group) or without (control group) BM-MSCs was inserted intraoperatively into the defect. The HSS knee scoring scale was used to measure the outcomes. At 16 months postoperatively both groups had significantly improved equally. At 42 weeks in the controls, there was irregular reparative tissue, little production of matrix and exposed subchondral bone. In the BM-MSC group, there was similarity to normal articular cartilage, and ECM was surrounding the repair tissue. Histologic and arthroscopic scoring systems were utilised to assess these findings. The stem cell group had significantly higher scores than the controls (75 % and 50 %, respectively).

In terms of improvement clinically, no difference was documented that was statistically significant. However, significant histological improvement was seen in the treatment group. The study sample however was of small size, as subjects at the stage of analysis were omitted (Wakitani et al. 2002). A different study by Grigolo et al. in 2009 divided rabbits in three groups (Grigolo et al. 2009). In the first a sham procedure was performed, in the second the ACL was divided and left untreated and in the third the transected ACL was treated with a Hyaff-11 BM-MSC (BM-MSC HA) scaffold on the left side and a plain scaffold on the right. In all specimens with ACL deficiency, OA developed. Repair tissue resembling hyaline cartilage containing intra- and extracellular type II collagen was seen in the BM-MSC HA group, without the presence of MMP-1 or type I collagen. Minimal amounts of type II collagen in the ECM and poor quality repair tissue were seen in the plain scaffold group, with the presence of atypical cartilage, type I collagen and MMP-1. In the stem cell group, minor irregularity of the articular surface and proteoglycan reduction was noted. The same occurred in the plain scaffold group, but to a higher degree.

BM-MSCs cannot be considered treatment for OA, as they are able to slightly slow down the degenerative process histologically, but not always clinically.

14.9 Conclusion

Various features of BM-MSCs render them excellent potential sport injury treatment. As opposed to different stem cell sources, BM-MSCs are not difficult to harvest with little morbidity, low anaesthetic demand and minimally invasive techniques. For instance, ACL fibroblast harvesting requires an arthroscopic procedure in a knee that has already been injured (Van Eijk et al. 2004). On the other hand, BM-MSCs can be obtained with aspiration from the iliac crest. It is not difficult to isolate BM-MSCs due to their potential to adhere to culture media easier than the rest of the cells of the bone marrow (Petite et al. 2000). Their potential to differentiate is not lost when cultured in vitro after isolation. Their capacity to differentiate towards various lineages makes them useful in the treatment of complex soft

tissue trauma. For instance, in the case of concomitant injuries of the femoral condyle cartilage, the meniscus and the ACL in the same knee, the application of BM-MSCs leads to their mobilisation to the injured tissues with a regenerative effect (Agung et al. 2006). Ethical issues and immunosuppression are overcome by their autologous origin.

Compared to amniotic fluid (AF-MSCs) and embryonic stem cells from the umbilical cord (EUC-MSCs), BM-MSCs are mineralised to a higher level during osteogenic differentiation. They can differentiate down the chondrogenic lineage as opposed to the infrequent differentiation of AF-MSCs and the barely existent EUC-MSC differentiation (Lovati et al. 2011). The BM-MSC properties are further amplified after their application. In physiological healing of wounds, BM-MSC activation is the rate-limiting stage and can be overcome by the implantation of preactivated stem cells with healing rate improvement (Kavalkovich et al. 2000).

The studies mentioned in the present review bring up several aspects of the subject. High BM-MSC concentration is required to further improve the outcomes. Most of the BM-MSC studies are performed in small animals, a fact that makes clinical application difficult given the individual human gait pattern and joint tissue biomechanics. Furthermore, the induced and clinical injuries are different. Thus, large animal or human studies are necessary to show clinical applicability.

Scaffold use is a potential element of any effective stem cell treatment according to the evidence provided in the studies above. The structural support that they offer and the prevention of cell leakage are major advantages. Nevertheless, isolated BM-MSC beneficial effects were documented as well.

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