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The regenerative therapies of the ankle degeneration; A focus on multipotential mesenchymal stromal cell application.

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Abstract

The ankle degeneration ranging from focal osteochondral lesions to osteoarthritis (OA) can cause a total joint function loss. With rising life-expectancy and activity of the patients, various regenerative therapies were introduced aiming to preserve the joint function via the induction of cartilage and bone repair. Here, biological events and mechanical changes of the ankle degeneration were discussed. The regenerative therapies were reviewed versus the standard surgical treatment. We especially focused on the use of multipotential **mesenchymal** stromal cells (MSCs) highlighting their dual functions of regeneration and cell modulation with the focus on the emerging MSC-based clinical studies. Being at an early step, more basic and clinical research is needed to optimize the applications of all ankle regenerative therapies including MSC-based method.

Key words:

Cartilage, Regeneration, Multipotential Mesenchymal stromal cells, Ankle, Osteoarthritis, cell therapy, Bone, Osteochondral lesion.

1. Introduction

A hallmark of the joint degenerative disease is the pathological involvement of different tissues mostly the damage of cartilage, as well as the thickening of the subchondral bones. These changes together cause pain and limitation or loss of the joint mechanical function [1]. Although frequently referred to as the 'ankle joint,' the linkage between the lower limb and foot consists of a complex of joint articulations. This complex comprises the talocrural (tibia-talar), subtalar (talo-calcaneal) and transverse tarsal (talar-calcaneal-navicular) joints. The talocrural joint contributes the broadest range of motion during gait and provides stability during the load bearing [2]. For this review, our referral to the degeneration of ankle has alluded to the talocrural joint.

Typically, the peak force experienced by the talocrural joint is of 4-7 times body weight, which is high compared to other joints of the lower limb and is carried over a much smaller contact area than the hip or knee [3]. However, the high level of joint congruency coupled with the differences in cartilage composition and structure between knee and ankle (i.e., higher proteoglycan with greater compressive rigidity in ankle) [4] explains why the ankle joint is less susceptible to primary **osteoarthritis (OA).** In contrast to knee and hip joints, the focal ankle osteochondral degeneration and **secondary** ankle OA are more common **[5]**. These degenerative ankle lesions are frequently caused by trauma, **e.g., fracture or chronic ligament instability** commonly because of the sport-related **activities.** Thus, most of these patients are young **individuals [6].** Several factors can predispose to the development of post-traumatic ankle OA such as genetic factors**, age, gender, muscle weakness, infection, and limb alignment [1, 7].** As **mainly affect younger individuals with longer expected lifespan, the ankle OA as a painful joint disease could markedly influence the patient quality of life. The physical function scoring of 196 patients with**

ankle OA has been reported as similar to or worse than that of patients with the end-stage renal disease or congestive heart failure [8].

No effective therapies are available to prevent the progression **of ankle degeneration** significantly**, and the joint replacement might not be the best option for young patients with ankle OA [6]. Thus,** the regenerative therapies aiming to preserve the tissues and function of the joint, have great potential for treating the young patients with ankle degeneration. Before considering the convenient type of the regenerative therapies for the ankle damage repair, it is essential to understand the two pathological events of degenerative ankle lesions; mechanical and biological and their **interplay (Figure 1). The knowledge of these mechanisms will** help to introduce or modify the therapeutic methods with best possible results.

2. Ankle joint degeneration and the ankle OA; tissues involved

Compared to knee OA, few animal models have been reproduced post-traumatic ankle OA only because age-related mouse models do not develop the ankle OA [9]. This trauma-like effect was performed via the resection of ankle-supporting ligaments creating three models according to excised ligament site, the medial-ligament model, the lateral-ligament model, and the bilateral-ligament model. The cartilage degeneration of talotibial was mainly demonstrated in the medial-ligament model. These mouse models have helped in describing the similarities between the **mouse and human ankles. For both human and mouse, the cartilage th**ickness in the ankle is about half that in the knee, and the density of ankle subchondral bone is higher than that in the knee **[9]**. **Another rat ankle sprain model has been developed by resection of lateral ankle ligaments with potential use for investigating the subsequent effect of cartilage degeneration [10].**

While focal ankle degenerative lesions involve mainly the hyaline articular cartilage and the subchondral bone, other tissues can also be affected such as synovium, and muscles in the ankle OA [11]. These pathological tissue changes often trigger ankle pain on weight bearing, and negatively affect the normal daily activities. In an inflamed joint, synovial cells and chondrocytes are major sources of a large group of immune cytokines; interleukin-1, -15, -17 (IL-1), (IL-15), (IL-17) and tumor necrosis factor-alpha (TNF-α). The increasing levels of these cytokines are associated with joint effusion and pain [12]. These inflammatory cytokines can interrupt the balance of the chondrocyte functions. Via the NFkB signaling, these cytokines stimulate the secretion of inducible Nitric Oxide Synthase (iNOS), Cyclooxygenase2 (COX2), and Prostaglandin E2 (PGE2) by chondrocytes. These changes are also associated with increased secretion of matrix proteolytic enzymes, metalloproteinases (MMPs). These together show that the inflammatory joint microenvironment is linked to the reduction in the synthesis and an increase in the degradation of the extracellular matrix collagen and ultimately the cartilage damage [13-15].

Bone alterations constitute an integral part of the ankle OA pathogenesis. The radiologically-detected changes of subchondral bone usually include subchondral bone sclerosis and osteophyte formation with associated severe cartilage damage [16]. These bone changes are also detected in the knee and hip OA [17] suggesting that these pathological bone responses are universal to OA and not ankle specific. Interestingly, the changes denoting the abnormal remodeling of bone in the ankle are usually concomitant with increased numbers of the bone resorbing cells, osteoclasts and high expression of the pro-inflammatory IL-1, IL-6, and TNF-α [18]. Additionally, the direct interactions between the bone forming cells, osteoblasts from subchondral tibia and femur, and chondrocytes lead to the reduction of proteoglycans and the induction of the MMP expression causing the cartilage damage in OA

[19]. Collectively, this confirms the direct relationship between the inflammation and the pathological bone changes as well as cartilage damage (Figure 1).

The weakness of the muscles within the joint can be correlated with the progressive cartilage damage as noted in knee OA [20]. Interestingly, the in vitro co-culture of muscle cells and chondrocytes enhances the resistance of the later cells to IL-1 and TNF-α effects [21]. Furthermore, the weakness of muscles could reduce the cartilage supportive roles of muscle cells, which are known to activate the collagen production by chondrocytes [22]. **Whether a similar effect of muscle weakness suggested for the cartilage damage progression in knee OA is not evident. However, an associated generalized atrophy of lower leg muscles detected in a radiological study of ankle OA suggested a consecutive rather than causative effect of muscle weakness [23].**

A connection between the obesity and the ankle pain is strong with the mechanical effect on skeletal alignment and muscle weakness could be critical contributing factors [24]. The intraarticular adipose tissues have a documented role in OA knee as shown in the experimental models or patients. The adipose tissues secrete high amount of the pro-inflammatory mediators, IL-1, and adipokines [25]. A strong correlation was reported between one of the adipokines, the Leptin and the MMP expression levels in the synovial fluid from knee OA patients [26] and between the mutation of Leptin and increase susceptibility to knee OA [27]. Similarly, another adipokine, Resistin also has a similar pro-inflammatory role via induction of PGE2 and suppression of proteoglycans [28]. **The effect of adipose tissue on cartilage showed for knee OA is less likely to be significant for the ankle OA. While in the knee joint, the infrapatellar fat pad lies in direct contact with the articular surfaces and cartilage [29], the ankle joint (talotibial) does not have such a fat pad. Thus, a similar pathological role for fat on cartilage in ankle OA is not expected.**

Altogether, different mechanisms in multiple tissues are participating in development and progression of ankle OA. The degenerative lesions involving mainly the cartilage and bone are usually manifested by pain and functional limitation of the joint.

3. Ankle joint degeneration and the ankle OA; Mechanical events

The stability of the ankle joint is determined both by the bony architecture and the soft tissue support [2]. The ankle sprains are one of the most common musculoskeletal injuries reported globally, accounting for more than 75% of all described ankle injuries [30]. Although several mechanisms could be involved in the ankle sprain, the most frequent is the lateral ankle sprain that involves inversion and excessive supination of the rearfoot during the landing/heel strike [31]. The severity of a sprain could range from a grade I classification, where the ligaments may be over-stretched, to a grade III sprain, where the ligaments are ruptured [32]. Arthroscopic studies of chronically instable ankles have identified the talar dome lesions associated with these conditions [33]. The osteochondral lesions of the ankle, associated with the sprain injury, are caused by a disruption between the cartilage and the underlying talar bone ranging from a small amount of local tissue bruising and up to an osteochondral fracture of the talus [34]. Due to the intrinsically small contact area of the ankle in a healthy joint, small changes to the biomechanics significantly alter the pressure of the talocrural joint and disrupt the local mechanics of the joint leading to adverse local contact conditions, such as increased shear stress within the cartilage resulting in its mechanical damage [33].

The ankle joint is also highly susceptible to the bone fracture, accounting for 9-14% of all fractures annually in the UK [35]. Altered biomechanics, disruption to the joint surface, and damage to the articulating cartilage may all lead to longer term degeneration of the ankle [36]. Imaging studies have identified arthritic changes in the ankle within five years following an inter-articular fracture. When progressed, patients with the ankle OA

demonstrate a significant change in the gait biomechanics compared with normal individuals. The off-loading compensatory mechanism employed by the patient may have some beneficial effect in reducing the shear stress within the cartilage. However, the biomechanical changes associated with the ankle OA also demonstrate a reduction in the ankle power and a substantial loss of muscle in the ankle region [37]. This may ultimately cause further mechanical changes locally within the joint as the function of the joint degenerates further and the bony deformity may arise in response to the mechanical changes.

4. The classic treatment of degenerative ankle lesions

Currently, the strategic choice of therapy for ankle degeneration depends on general factors such as age, health as well as the symptom severity, joint function, and the degree of skeletal tissue degeneration [1]. The first line of treatment for early and mid-stages of ankle OA is conservative to treat the pain and help to delay the need for the surgery. The conservative treatment is also the primary option for patients who do not qualify or with contraindications for joint sparing surgeries or ankle replacement. These conservative methods include dietary supplementation of glucosamines, visco-supplementation, **Platelet-Rich Plasma (PRP),** the ankle-foot orthoses (AFO), and physiotherapy. A daily oral dose of glucosamine sulfate has proven to be **safe and significantly reduce the symptoms of lower limb OA, particularly knee [38]. However**, more studies confirming the glucosamine effectiveness on ankle arthritis are needed. The visco-supplementation via the local injection of hyaluronic acid (HA) could facilitate the movement and help to release the pressure on the joints. Additionally, **HA binds to CD44 molecule on the surface of synoviocytes and chondrocytes suppressing the expression levels of pro-inflammatory cytokines, MMPs and prostaglandins [39-41].** In a recent clinical study, the local injection of PRP into ankle has been reported as a safe procedure and helps to delay the need for surgical intervention

[42]. The AFO is used for the ankle to restore static and dynamic foot alignment, and to reduce the pain. **However, the AFO should be used thoughtfully as it could cause movement restriction [43, 44].**

When symptoms are persisting and with the progression of OA, one of the several surgical therapies can be chosen for the patient (Table 1). Osteotomy is a procedure used to correct an axial malalignment, a partial joint degeneration, or fracture mal-union [45]. The basis of osteotomy is resuming the alignment of the joint into normal contact areas of the articular surfaces and accordingly correcting the forces applied to the ankle joint. Several studies showed good results using the ankle osteotomy, but it has been reported that the symptoms and signs of synovitis can worsen over time [46]. Additionally, this procedure cannot be used at the end stage the ankle OA or in the old patients with low bone quality [47]. Although no biological boosting is included in this method, it becomes frequently applied in association with some regenerative approaches as described below.

In the late stage of degenerative ankle lesions, arthrodesis is still considered as the gold standard surgical therapy. This procedure aims to fix the ankle bones by performing a fusion between the tibia and talus and this usually help to improve the weight-bearing activities of the ankle joint [48]. But arthrodesis could have low-cost effectiveness due to present risk of non-healing with the need for a second surgery and the high risk of developing OA [49]. In contrast to arthrodesis, ankle replacement is increasingly used mainly for the old and less active patients with severe joint damage [50]. However, ankle replacement could be associated with various post-operative complications such as instability, loosing, failure and periprosthetic fractures [51]. For both treatments (arthrodesis and ankle replacements), the criteria for the patient selection are a powerful tool that can make considerable differences in the complication and re-operation rate, but the activity restriction is still the main disadvantage for ankle fusion in particular. In summary, although variable choices are

available, the clinical outcomes of the surgical treatment are not always successful. Furthermore, the cadaveric studies have highlighted that clinical intervention does not fully restore the native ankle joint contact or the hind-foot mechanics [52] indicating that new improved therapies correcting the biology in addition to the mechanics of ankle are still required.

5. Regenerative therapies of degenerative ankle lesions

Different regenerative methods have been recently introduced aiming mainly to repair the cartilage tissues, delaying progressive bone changes, and avoid surgeries involving a restriction in the joint movement. These methods either include the grafting of bone/cartilage tissue or cultured chondrocytes or the stimulation of existing chondrocytes, i.e., osteochondral grafting, autologous chondrocyte implantation or arthrodiastasis respectively. Other methods work by concentrating and activating the resident bone/cartilage progenitor cells; **mesenchymal (or multipotential)** stromal cells (MSCs) using the microfracture technique or by external implantation of MSCs into the joint. **These cell-based therapies usually aim to promote cartilage healing and probably limit the inflammatory response (Figure 1).**

i. Osteochondral grafting

The osteochondral grafting or mosaicplasty involves the use of an osteochondral cylinder graft harvested from low-demand site, e.g., knee intercondylar region or lateral trochlea [53] or from the ipsilateral talar articular facet [54]. This method can be used for the lesions up to 2 cm^2 and it has been reported with promising results showing tissue regeneration and improvement of the joint symptoms [53, 54]. However, the limitation for the osteochondral grafting is usually the pain and bleeding at the donor site [55]. Additionally, little evidence

about the graft integration and survival has been shown with high rates of long-term clinical failure with need for a second surgery [56]. Together, alternative use of cells instead of tissues could be a better regenerative option as described below.

ii. Autologous chondrocyte implantation

Autologous chondrocyte implantation (ACI) involves the seeding of culture-expanded chondrocytes then locally implanting into the joint [57]. Although arthroscopic ACI has been used in the ankle with satisfactory effects [58], this technique still has several limitations including inadequate functions of chondrocytes particularly in the old individuals and the large-size cartilage defects. Additionally, the cost, the long-term preparation and the in vitro manipulation and the need for two surgical procedures are hard to overcome [59]. Interestingly, an osteochondral plug can be generated in vitro by combining the collagen-based matrices seeded by human chondrocytes with devitalized sponge cylinders using a fibrin gel [60]. Additionally, an engineering of the chondrocytes could be a potential option targeting specific molecules such as miRNA-140 that has a regulatory effect on the expression of MMPs [61]. Such modifications in ACI can further improve the clinical results, but the donor age and the lesion size are essential factors still to be considered.

iii. Arthrodiastasis

Arthrodiastasis or the joint distraction is used in the young patients having normal alignment of the ankle joint [62, 63]. It includes using an external ring fixator together with performing a gradual distraction up to 5 mm. The rationale of this method is the activation of self-regenerating abilities of the osteochondral tissue via differentiation of MSCs. When the joint is distracted, the resident synovial fluid-MSCs favorably adhere to the cartilage in distracted joints due to the reduction of synovial hyaluronic acid as shown in an experimental model of

knee OA [64]. Additionally, the alterations in intra-articular hydrostatic fluid pressure during arthrodiastasis are believed to stimulate the chondrocytes to produce proteoglycans and collagen helping the cartilage repair [65]. The clinical outcomes of the ankle arthrodiastasis have been reported as satisfactory with pain reduction, fibrocartilage formation, and decreased subchondral bone thickness indicating an improvement of the bone remodeling [65]. Nevertheless, this procedure could be associated with some complications such as infections, neurovascular injuries, damage to ankle ligaments and repair failure because of the patient non-compliance [66]. Together, arthrodiastasis has a regenerative value by different mechanisms activating intrinsic MSCs and chondrocytes, but more studies are still required to minimize complication risk.

iv. Microfractures

Microfracture repair of the articular cartilage lesions involves drilling holes in the subchondral bone together with the removal of the calcified cartilage parts [67]. As experimental research has shown that bone marrow-MSCs could help the cartilage repair, this technique aims to cause the bone marrow bleeding that then forming a clot that contains local MSCs to maintain the cartilage regeneration [68]. This microfracture technique has been used successfully particularly for young patients having small talar osteochondral lesions and mild osteosclerosis with substantial functional improvement [53, 69]. However, this procedure seems to be ineffective in talar osteochondral lesions larger than 1.5 cm^2 [70]. Additionally, the history of trauma and the presence of osteophytes and unstable osteochondral defects are usually indicative of the poor outcomes [67, 70, 71]. The effect of the patient age on microfracture **outcomes is controversial [68, 71].** The location of the **degenerative lesion could affect on the microfracture outcomes, for** example, the osteochondral lesions of lateral talus has been found to be correlated with positive functional **results [72].**

In summary, although osteochondral grafting, ACI, arthrodiastasis and microfracture deliver satisfactory clinical outcomes over midterm follow-up stages, these methods frequently fail in the long term. **To improve the outcomes of these regenerative methods, scaffolds can be implanted to support the tissue healing. Smart scaffolds made of multi-layered biomimetic structures have been reported to induce the formation of both bone and cartilage [73, 74]. In sheep model with osteochondral defects, bone and cartilage regenerative results were reported for those scaffolds that were used with or without autologous chondrocytes, suggests that the main mode of action of the scaffold is based on the recruitment of local cells [73]. A clinical study involved 30 patients with knee chondral or osteochondral lesions, has shown that smart nanostructured scaffolds fabricated of type I collagen and hydroxyapatite scaffold could promote cartilage and bone healing together [74]. For degenerative ankle lesions, studies are needed to test the efficacy of such smart scaffolds when combined with the microfracture technique for promoting the cartilage healing.**

v. Implantation of MSCs

Source, function and rationale for the use of MSCs

MSCs are widely existed within the musculoskeletal, the fat and synovial tissues and the synovial fluid. Although the in vivo markers are not fully defined and appear variable, MSCs are well-characterized in vitro after the culture-expansion by being plastic-adherent, expressing the surface markers, CD90, CD73, and CD105, but are not expressing hematopoietic lineage markers [75]. These cells have the multi-lineage differentiation capacity (including bone, cartilage, and fat) making them suitable for the ankle osteochondral tissue repair [75]. Additionally, MSCs could affect other cells particularly immune cells via the secretion of immunomodulatory mediators [76].

The therapeutic value of the MSC administration into joint has been primarily shown using preclinical OA knee models, where cartilage repair and increased type II collagen were documented [76]. **In addition to cytokines and growth factors, MSCs can produce proteins that are important for the cartilage tissue formation and maturation such as collagens, fibronectin, glycosaminoglycan, and proteoglycans as shown using ex vivo cells [77]**. In addition to the tissue regeneration, MSCs can respond to the local environmental signals such as cytokines and growth factors that are produced in response to tissue injury [76]. A good example for the paracrine effect of MSCs is their ability to suppress the proliferation and the effector functions of both innate and adaptive immune cells. This immunomodulatory effect could be of great benefit controlling the inflammatory response and preventing the inflammation-related tissue damage in the ankle OA [78]. Because of these beneficial properties, MSC implantation into the ankle joint has been employed in several studies, as discussed below.

MSC-based clinical studies of the ankle OA

Several clinical studies have reported the use of MSCs with success particularly when compared with one approach of the standard surgical treatment (Table 2). The ankle OA and focal osteochondral lesions, as well as ankle bone non-union have been targets for the MSC implantation in several studies during the last two decades. A large study assessing the adverse effects of using autologous bone marrow concentrates or culture-expanded MSCs in several cases of orthopedic patients including the ankle OA has demonstrated no risk of the application in human [79]. In another study, using a single injection of autologous bone marrow-derived culture-expanded MSCs in moderate to severe cases of the ankle OA has been shown to be safe with beneficial outcomes of the pain reduction, the functional improvement and the cartilage repair [80]. An ongoing clinical trial involving MSCs is a

investigating the side effects of intra-articular injection of cultured autologous bone marrow MSC in patients with ankle OA within six month-follow up **(https://clinicaltrials.gov, NCT01436058).**

Instead of the use of culture-expanded MSCs, **the bone marrow concentrate (BMC) could be a source of native MSCs, which have promising outcomes as a regenerative cell-based therapy helping the treatment of ankle OA or osteochondral lesions. This application of these native MSCs helps to avoid the complicated steps and high costs of ex vivo culture of MSCs [81]. Additionally, application of bone marrow concentrate could have another advantage as containing soluble factors helping cartilage and bone repair [82]. These factors include growth factors such as Transforming growth factor beta (TGF-ß), BMP-2, VEGF and Platelet-derived growth factor (PDGF) and inflammatory cytokines IL-1 and IL-8 [83].**

Hernigou et al. showed that percutaneous injection of bone marrow MSCs within bone marrow concentrate for ankle non-unions could enhance the bone regeneration better than using iliac crest-harvested bone autograft [84]. Moreover, Hauser et al. reported in a case study, that the multiple local deliveries of whole bone marrow in conjunction with hyperosmotic dextrose for the treatment of the ankle OA, can reduce the pain and improves the joint function [85]. To provide a matrix for regeneration MSCs, Buda et al. used a concentrate of autologous bone marrow seeded on scaffold and enriched with platelet-rich fibrin (PRF) as a source of growth factors (such as TGF-β) for osteochondral lesions of the talus. Their results proved that this combination of biological factors could improve the cartilage healing process as good as ACI and have an advantage of one step procedure instead of two [86]. Similar to PRF, platelet rich plasma (PRP) is a biological material containing a multitude of platelet-derived growth factors, chemokine, and immunomodulatory molecules with beneficial effects on MSC repair cells can be used clinically to enhance the MSC

proliferation and differentiation properties [87]. In other two studies, patients with focal talar osteochondral lesions were treated with bone marrow concentrate seeded onto collagen scaffold or hyaluronic acid membrane in comparison to ACI technique. The clinical and radiological outcomes of both therapeutic strategies were similar with hyaline cartilage formation over long-term follow-up [88, 89].

In addition to bone marrow, adipose tissue was used as a source of MSCs. Injection of autologous adipose tissue-derived MSCs (after removal of other fat and connective tissue cells) into the ankle joint combined with microfracture has been shown to induce better cartilage repair when compared to the microfracture method only [90]. **Another study is currently testing the safety and efficacy of autologous adipose-derived stromal vascular fraction that contains MSCs for the treatment of OA of different joints including hip, knee, thumb, and ankle joints (**https://clinicaltrials.gov**, NCT03166410).**

The synovial fluid could be another potential source of MSCs because MSCs derived from synovial fluid of ankle with osteochondral lesion showed similarity in phenotype compared to bone marrow-MSCs with a preserved capability of differentiation [91]. Regardless the MSC tissue source, using autologous rather than allogenic MSC-based therapies seem to predominate for therapy of the ankle OA, presumably due to the relatively young age of the patients with fewer concerns regarding their therapeutic efficacy that could deteriorate with aging.

Challenges in MSC-based therapies

Although autologous MSCs used in clinical trials had success with the advantage of less cost and manipulations, none of the above studies have investigated the immune modulatory role of MSCs that could influence the pro-inflammatory cytokines (IL-1, IL-15, IL-17, TNF-α)-related cartilage damage. Also, the expression levels of these cytokines were not measured

after MSCs implantation. Hence, whether the positive clinical outcomes of the MSC use are related to the differentiation, secreted growth factors, regulation of immune response or combination, is still unclear. Although has no clinical evidence, the MSCs' tumorigenic potential has been shown in experimental models because MSC-mediated immunosuppression and anti-apoptotic mechanisms could promote tumor progression **[92]**. This tumorigenic potential suggested that the immunomodulatory effects of MSCs should be carefully assessed mainly in patients with immune disorders and cancer.

Although BM concentrates has many the advantages of, the doses of delivered MSCs in bone and cartilage degenerative lesions such as OA, remain poorly known and controlled. The low frequency of BM-MSCs among other BM cells together with wide variability related to the aspiration techniques and donor age and gender [93-95] could lead to variable outcomes of this therapy. Thus, it is important to determine the MSC quantity needed for satisfactory results similar to what has been shown for the healing of the fracture bone non-union [96]. We have reported recently how an assay using a flowcytometry to phenotype and quantify MSCs, could be a suitable tool to indicate **the the effectivness of BM concentrates used for clinical regenerative applications [97].**

Assessing the functionality and the survival of implanted MSCs is an important factor to be considered for therapy. Campbell et al. reported that in the tissues of hip OA patients, CD271+ endogenous MSCs within the subchondral bone tend to accumulate in the areas adjacent to cartilage defects [98]. These MSCs appear to be less proliferative and have lower mineralization capacities as well as a high expression of CXCR1 and CCR6 chemokine receptors indicating of their altered migration capabilities [98]. The functionality of autologous MSCs in the damaged areas of cartilage and bone in ankle OA remains to be further explored. Additionally, to compare their competency with donor-matched bone marrow and adipose MSCs. These data could explain the failure of tissue-resident MSCs to

repair the ongoing osteochondral damage in OA and as such, provide a strong rationale for the addition of extra 'healthy' MSCs to the defect sites or using allogeneic MSCs for these elder patients. The mechanisms of alterations of the MSC function at the sites of the damage remains uncertain, but have been linked to altered biomechanical signaling from mechanical-sensor cells, osteocytes to MSCs leading to perturbations in their osteogenic differentiation and aberrant bone remodeling as shown in knee OA [99]. It is yet to investigate if similar processes are taking place in the ankle OA.

Collectively, all these data indicate that the induction of cartilage tissue repair using MSCs should go together with the consideration of interactions with local biological microenvironment and mechanical factors. Despite encouraging results from the MSC-based clinical trials, many questions remain unresolved, particularly for the MSC survival and mechanisms of action upon implantation. Additionally, testing the effect of the mechanical correction when combined with the MSC implantation is essentially needed.

6. Future perspective

The complexity of the pathology of ankle OA with the focus on the cartilage repair could be a reason for the limited or the short-term effects of current regenerative therapies. Several changes such as muscle weakness or injuries and adipose tissue changes are influencing the cartilage and bone damage in OA. Thus, the repairing and restoring the functions of other joint tissue should be considered. Additionally, the combination of these regenerative therapies and the correction of mechanical loading following could have a significant advantage of the long-term regeneration of the damaged cartilage. The mechanical ankle correction should aim collectively to treat the bone abnormalities and to strengthen the muscles/ligaments. A direct link between inflammatory mediators and loss of chondrocyte functions has been shown. However, further molecular studies are needed to examine how

targeting inflammation could improve the cartilage repair therapies. Altogether, treating ankle degeneration should be planned for a whole joint and not only cartilage-specific.

MSCs have the great potential to be a favorite reparative method for the ankle degeneration. But, again these cells should be used as a part of a combined therapy. Critically, the response of MSCs particularly in OA patients towards the mechanical stimulation is complicated and could affect the clinical outcomes. Therefore, further understanding of the signaling pathways initiated in response to the mechanical stimulation in MSCs is essentially required. This knowledge will help to optimize the use of MSCs for effective cartilage engineering. The use of matrices such as the collagen scaffold or the hyaluronic acid membrane is of great value to provide mechanical stability and consequently support the MSC differentiation and paracrine functions. Similarly, adding another therapeutic element via using the biological stimulators of MSC function such as PRP is highly recommended using biological factors. Similar to the diamond concept of bone repair [100], a combination of the progenitor cells (i.e., MSCs), scaffolds, growth factors, and corrected mechanics could be applied as a one combined modality for therapy of ankle focal osteochondral lesions or the ankle OA. However, implementing this concept would require further development of the suitable pre-clinical in vitro and animal models where the implantation of these factors could be reliably be tested individually and in combination.

Uniquely, in addition to the mesenchyme differentiation capacity, MSCs can modulate surrounding cells and microenvironment by releasing various cytokines, growth factors, and chemokines. While tissue regenerative and paracrine functions of MSCs are strongly demonstrated in vitro, the phenotype, topography, and function of in vivo MSCs in the ankle OA is not clear and needs further investigations. Another consideration for the MSC-based therapy for the joint degeneration is the heterogeneity of MSCs with regards their abundance and the differentiation potential, particularly between donors. Thus, determination

of the optimal quantity given for therapy is necessarily required. Also, the search for new specific markers to select the best functioning MSCs is needed. If defective, an alternative solution could be the induction of a particular function of MSCs using the cell engineering technologies. This approach could aim to express the extracellular matrix proteins and the growth factors by MSCs or harnessing the cellular machinery that controls the MSC differentiation capabilities. Together, more experimental, and clinical studies are still required in parallel to deliver the best regenerative method for the joint degeneration in general and the ankle in particular.

Executive summary

- **Ankle joint degeneration /OA**
  - ❖ The pathological events of ankle local osteochondral lesions or OA usually involves both mechanical and biological elements, which could lead to progressive loss of joint function, especially in young patients.
- **Classic treatment of ankle degeneration/OA**
  - ❖ Using surgical solutions such as joint replacement or arthrodesis for treating degenerative ankle lesions have high costs with long-term complications particularly with increasing patient life-expectancy and activity.
- **Regenerative therapy of ankle degeneration/OA**
  - ❖ The challenges of current therapies demonstrate the essential need for regenerative treatments as alternative options preserving the joint tissue, controlling inflammatory joint environment, and maintaining natural mechanical functions.
  - ❖ The regenerative treatment should be planned to take in account correction of various mechanical and biological pathological elements.

- **The clinical use of osteochondral tissues or mature chondrocytes**
  - The various regenerative therapies involving the grafting of osteochondral tissues or implantation of mature chondrocytes (ACI) are still evolving, but have some limitations mostly related to tissue harvesting and/or costs.
- **Arthrodesis and Microfractures are other examples of regenerative therapies mainly dependent on activation of resident MSCs to promote the cartilage healing.**
- **The rationale for clinical use of MSCs in therapy of ankle degeneration/OA**
  - The clinical use of MSCs has a great potential for regenerative ankle therapy as MSCs can act as progenitors for cartilage and bone as well as being cell modulator influencing inflammatory microenvironment.
  - Although of limited popularity compared to the knee joint, MSC-based clinical studies in degenerative ankle lesions have promising results that are comparable to those of other regenerative methods.
- **Challenges and considerations for clinical use of MSCs**
  - Biological factors should be considered for MSC-based therapy including the source and the donor-dependent function and numbers. Also, extent and co-existence of specific biological signals such as TGF-Beta could affect the therapy outcomes.
  - The MSC chondrogenic differentiation can be particularly affected by the mechanical loading types and these factors can affect the therapeutic outcomes of MSCs.
  - To boost the results, the design of more effective clinical trials involving MSCs for treatment of the ankle degeneration could also include scaffolds and a biological modifier such as platelet growth factors. Finally, the mechanical forces constitute a vital element to be considered.

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** These three studies have demonstrated that the application bone marrow concentrates seeded on scaffold was as effectiveive as ACI for treating osteochondral talus lesions.

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** This study has shown that the microfracture technique combined with local injection of adipose-MSCs was more effective than microfracture only as for treating ankle OA in 64 patients.

Figure 1: The mechanism of development of ankle OA and the proposed mechanism of action of cell-based therapy.

Both biological and mechanical events are involved in the development of ankle OA. The mechanical injuries of the ankle such as sprain or fracture can shear stress and consequently cause bone changes as well as cartilage damage. Also, inflammatory cytokines released by inflammatory synovium and chondrocytes can, in turn, change the chondrocyte function and induce MMP production. Bone alteration is presumably can promote the secretion of MMPs when bone cells interact with chondrocytes. The cell-based therapy involving MSCs aims to prevent further cartilage damage/help repair and suppress the inflammation.