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ORIGINAL ARTICLE

Age-associated changes in regenerative capabilities of mesenchymal stem cell: impact on chronic wounds repair

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Abstract

Mesenchymal stem cells (MSCs) represent an ideal source of autologous cell-based therapy for chronic wounds. Functional characteristics of MSCs may benefit wound healing by exerting their multi-regenerative potential. However, cell ageing resulting from chronic degenerative diseases or donor age could cause inevitable effects on the regenerative abilities of MSCs. A variety of studies have shown the relationship between MSC ageing and age-related dysfunction, but few associate these age-related impacts on MSCs with their ability of repairing chronic wounds, which are common in the elderly population. Here, we discuss the age-associated changes of MSCs and describe the potential impacts on MSC-based therapy for chronic wounds. Furthermore, critical evaluation of the current literatures is necessary for understanding the underlying mechanisms of MSC ageing and raising the corresponding concerns on considering their possible use for chronic wound repair.

Introduction

Chronic wounds or non-healing wounds remain a clinical challenge in medicine and represent a significant health burden in modern society (1). While standard therapies, including debridement, pressure offloading, dressing regimens, hyperbaric oxygen, antibiotics and topical growth factors, have improved management of wounds and relatively shortened the healing time, there are few therapeutic approaches that effectively reverse the consequence of fibrosis or scar. As the general population continues to age, the number of patients with diabetes and other chronic ageing-related diseases is growing dramatically (2); hence the need for more effective approaches to treat many chronic diseases, including non-healing wounds becomes more important. One promising solution, cell therapy, involving the transplantation of progenitor/stem cells to patients through local or systemic delivery, has heralded a new area of research for the treatment of wounds with delayed healing (3).

Key Messages

- chronic wounds are an immense social burden in the ageing society
- mesenchymal stem cells (MSCs) with unique regenerative potential serve as a promising treatment for non-healing wounds
- the ageing-related changes in MSCs would certainly affect their regenerative capabilities in chronic wound healing
- the underlying intrinsic and extrinsic mechanisms and corresponding solutions are still limited
- more attention should be paid on improving regeneration potential of ageing MSCs in the treatment of chronic wounds

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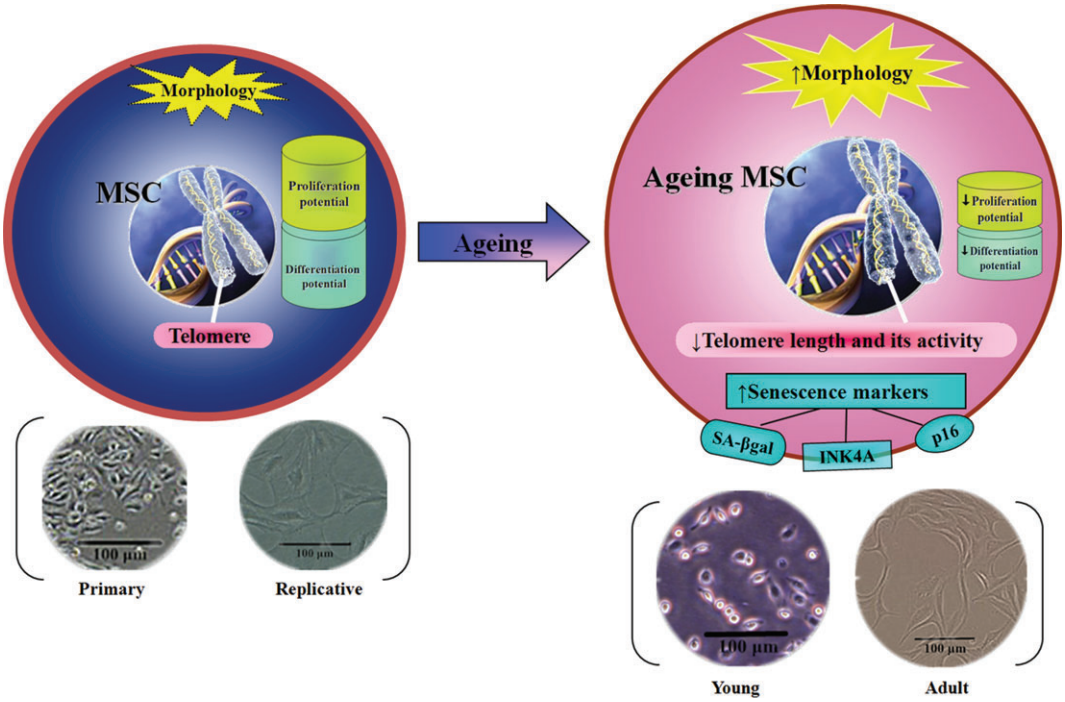

Figure 1 Ageing-induced alternations in mesenchymal stem cells in terms of morphology, proliferation potential, differential potential, telomere and senescence markers.

Mesenchymal stem cells (MSCs) are of great interest because of their unique regenerative potential. The beneficial effect of exogenous MSCs on chronic wound healing had been shown in a variety of animal models and in reported clinical cases (4). However, there appears to be an inevitable link with ageing-associated impact on MSCs and their regenerative capabilities on chronic wound healing. Accumulated data indicate that MSCs from elderly donors or long-term ex vivo cultivation show great difference compared with that of young donors in cell morphology, proliferation potential, differentiation potential, telomerase length and activity, as well as special molecular markers (5,6). Moreover, several researchers found that passages of in vitro culture share equal importance with donor age when considering the proliferative and differential properties of MSCs (7,8). The decline of regenerative capacity of MSCs is predicted to be caused by cellular ageing (9). Thus, regenerative potential of MSCs might change with age or cultured passages, which suggests a possible limitation in their use for chronic wound repair.

In this review, the ageing-associated changes in MSCs and the related molecular mechanism is summarized. In particular, the impact of these changes on the regenerative capabilities of MSCs and current improvements is described, specifically for chronic wounds.

Age-related changes of MSC features

Age-related changes in MSCs not only mediate cell morphology, proliferation and differentiation, but they also impact on the telomere length, telomerase activity and cellular senescence markers (Figure 1).

Cell morphology

After 20–30 population divisions, MSC morphology shows obviously larger (10) and wider alterations, and the proliferative ability becomes more slow (11) than that of young counterparts. The alternations in cell morphology are typically associated with the Hayflick limitation of cellular senescence (12). Bonab (13) observed that the cytoplasm became granular with many cell inclusions, and debris was formed in the medium after 3 months of in vitro culture. Aged MSCs show reduced numbers of spindle-shaped (young) cells in culture, which is exhibited in very early stage of cultivation in MSCs from young donors and is gradually lost with increased cultivation time (10). Notably, transfecting simian vacuolating virus 40 (sv40) (14) or telomerase (15) into MSCs considerably reduces the cell sizes compared with their initial sizes.

Proliferation

The decrease in proliferation capability of ageing cells is manifested in MSCs. Hayflick (16) observed that somatic cells division happens for a limited number of times. The maximal in vitro population doubling of MSCs is 30–40, while embryonic stem cells (ESCs) show no such loss (17,18). MSCs from aged donors show a significant decrease in the growth rate (19) and decline in replicative lifespan of ageing somatic cells (20). These results suggest that proliferative capacity is gradually lost with increasing cultured time and donor age.

Differentiation

The capability of MSC differentiation appears to change with age in some cases (17). In chondrocyte induction test, the

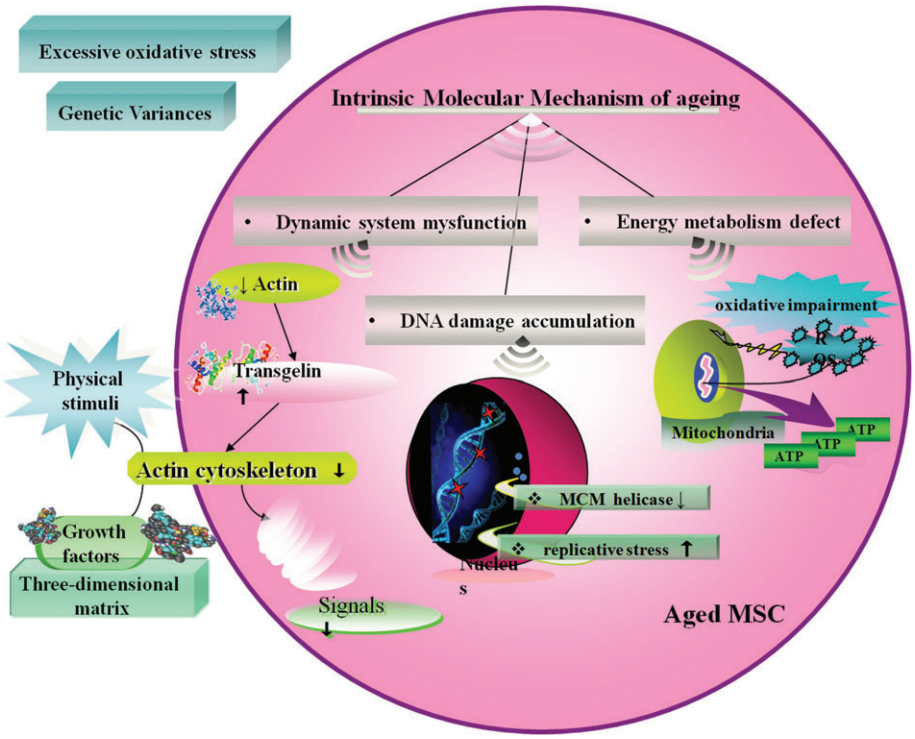

Figure 2 Intrinsic and extrinsic molecular triggers for mesenchymal stem cells ageing.

specific genes SOX9, COL2A and AGG sharply decreased with donor age (21). However, the age of the MSC donors had little effect on the ALPase activity or the calcium content for osteogenesis induction and glyceraldehyde-3-phosphate dehydrogenase (GPDH) activity on oil red O staining for adipogenesis induction (22), while another study reported the age-dependent changes of MSCs in relation to the osteogenic potential (23). In addition, with the increased culture time in vitro, the homing ability of transplanted MSCs was severely reduced (24).

Telomere length and telomerase activity

Telomere shortening was found in almost all the ageing cells and was thought to be the first possible mechanism of cellular senescence (25). Cells generally lose proliferation capacity and enter senescence once the telomeres reach a certain length (26). It is reported that telomere shortening happens in MSCs at the rate of 100 bp per every two passages, and in early to late passage cells, the telomere lengths decrease from an average of 10·4 to 7·1 kbp (8). On this basis, the correlation between the dividing capacity and telomere length was studied, in both in vitro and in vivo stem cell ageing (27).

Proliferation was greatly related to telomerase activity but telomerase activity is markedly decreased in somatic cells and other non-embryonic stem cells such as progenitors after 30–40 population doubling (28). Although many studies have detected telomerase activity in MSCs (29), whether MSCs possess telomerase activity is controversial. The divergence in various results obtained in MSCs is due to varying donor age and species (18). Other researchers state that forced telomerase up-regulation could increase the division times and enhance differentiation potential in human MSCs (30), suggesting that telomerase activity may play a vital role in maintaining the proliferative and differentiation potential of MSCs, and its dysfunction leads to the MSC ageing process.

Cellular senescence markers

The first identified senescence marker is senescence-associated β-galactosidase (SA-βgal); its activity reflects the growing lysosomal compartment that usually occurs in aged cells (12). Recently, more senescence markers were identified: p16 ^{INK4A}, DEC1, p15 INK4B and DCR2 (31) as well as cytological markers: senescence-associated heterochromatin foci (SAHFs) and senescence-associated DNA damage foci (SDFs) (32,33). SDFs are abundant in proteins that are related to DNA damage and exist in senescent cells from both mice and humans. Expression of genes that promote cell cycle progression (i.e. c-FOS, cyclin A, cyclin Band PCNA) (34) is suppressed in ageing cells. In the aged MSCs, apoptosis-associated proteins such as p53, caspase 3, caspase 8, caspase 9 down-regulated and Bcl-2, which enhance survival of many cell types, are expressed at a high level (35).

Mechanism of MSC ageing

Although the beneficial effect of exogenous MSCs on chronic wound healing had been evidenced in pre-clinical and clinical studies, autologous treatment with MSCs from older donors appeared to be less effective than application of their younger counterparts (36). These age-related differences remain incompletely understood, as do their functional consequences. Thus, exploring the mechanism of ageing MSCs is vital to indicating age-related MSC impacts on chronic wound healing (Figure 2).

Intrinsic molecular mechanism

Dynamic system mysfunction

Actin turnover significantly decreases in ageing MSCs, leading to the up-regulation of one actin cross-linking protein, transgelin, also the biomarker of ageing, which reduces the actin cytoskeleton dynamics. Actin cytoskeleton translates and processes the external physical stimuli or biochemical molecules into intracellular signals through various growth factors and three-dimensional extracellular matrix structure. Considering this, the actin cytoskeleton could not respond adequately to these factors and was less dynamic. For instance, the mechanical signals regulating bone homeostasis and regeneration are transduced via actin cytoskeleton (37), which may be responsible for the frequent bone-related diseases in elderly patients. By contrast, it is intriguing that mechanical requirements appear to change with age in vivo (38). Thus, lower actin dynamics result in regenerative potential decrease of senescent MSCs due to slower and less response to the environmental signals, both biological and mechanical (39).

Energy metabolism defect

Almost all aged cells suffer mitochondrial damage and oxidative impairment (40). Mitochondria are the central organelle supplying energy in the form of ATP. Energy generation, which is accompanied by unstable reactive oxygen species (ROS), may damage both the mitochondrion itself and other components of the cell, which finally causes ageing as a result of damage accumulation (41). Mitochondria are essential for all cells in life and death, and energy support is fundamental for cell differentiation (42). In adult MSCs, early passage cells contain more undifferentiated stem cells that form a significant cluster of mitochondria around the nucleus (43). Frequent self-renewal of stem cells requires more mitochondria resistant to the cellular senescence.

In aged MSCs, expression of several proteins involved in antioxidant defence up-regulate along with decreased antioxidant power, which appears to occur in other cells, such as the increased expression of peroxiredoxins in mouse embryonic fibroblasts with age (23). This might expose cells to higher ROS levels, as well as age-dependent reduced metabolic activities, resulting in insufficient capacity to defend effectively. Thus, despite decreased antioxidative proteins not being the cause for MSC ageing, the molecular damage results from reduced ROS elimination capability which may induce cell dysfunction over time (44).

DNA damage accumulation

Haematopoietic stem cells (HSCs), another kind of multi-potent stem cell, show a dramatic decline in regenerative function activity with age, resulting in degraded blood production and impaired engraftment following transplantation. Flach (45) demonstrated that cycling old HSCs in mice has heightened levels of replication stress associated with cell cycle defects and chromosome gaps or breaks, which are due to decreased expression of mini-chromosome maintenance (MCM) helicase components and altered dynamics of DNA replication forks. As HSCs share several features with MSCs, such as various differential capability and self-renewing ability, we assume that the DNA damage accumulates because the high replicative stress is a potential driver of functional decline in MSCs from aged donors.

Extrinsic molecular mechanism

Excessive oxidative stress

Cells cultured in vitro enter senescence after a certain number of cell divisions. Telomeres can shorten during expansion and excessive oxidative stress can cut down the rate of telomere loss (46). Ageing is considered as a complicated stress response triggered by activation of three main mechanisms: telomere erosion, DNA damage and INK4/ARF locus repression. All of these pathways relate to the tumour suppressors p53 and RB, and are highly consistent with the production of oxidative stress during cell culture, named stress-induced premature senescence (SIPS) (47,48).

Genetic variances

Complete characterization of expanded MSCs is essential in serial passages as ageing imposes restriction on MSC application; especially MSC-based therapies have achieved some success in many disease treatments (49,50). Cai (51) performed a whole-genome sequencing of MSCs in ex vivo culture to locate the genetic variances. There are no obvious changes in copy-number variation and low levels of single-nucleotide changes (SNCs) in the initial phase but a significant number of SNCs is found in passage 13. In primary culture and early passage, MSCs had low possibility of SNC mutations but reached a high frequency in passage 13, which clarifies that genomic composition of ex vivo MSC cultures tends to be unstable with extended expansion and may be responsible for the ageing process.

Environmental influence from chronic wounds on MSCs

Apart from the systemic ageing, chronic wounds and their pathological microenvironment may negatively affect MSCs itself and their positive properties, and impaired repairing properties may diminish the effectiveness of autologous cell therapy in patients with chronic wounds (52). For example, in the case of chronic wounds in diabetic patients, the high level of glucose induces up-regulation of BAX in MSCs which encourages apoptosis of the stem cells before differentiation or proliferation (53). On the other hand, the advanced glycation end products in the wounds of diabetic patients could inhibit the level of Bcl-2 and increase the caspases, FAS and BAX to promote cell apoptosis by giving rise to higher oxidative stress. Besides, cells near the wound could secrete cytokines such as IL-2, IL-4, IL-7 and so on inducing MSC apoptosis by suppressing Bcl-2 expression. The dysfunction of TGF-β1 up-regulation after impaired and over-expression of TGF-β3 in diabetic ulcers also causes limitation in MSCs to prevent their repair capability (54,55). While ample evidence exists that microenvironment of chronic diseases severely affect MSCs in the regenerative capability,

further investigation on their interaction and mechanisms is important for effective clinical application.

Possible impacts of ageing in MSCs on chronic wound healing

The application of MSCs in cell therapy is being studied in several areas of medicine, including chronic wound healing. Badiavas *et al*. directly injected autologous bone marrow derived MSCs (BM-MSCs) to the edge of a wound, and achieved complete closure of the wound (56). Dash and Lu treated 24 patients having chronic ulcers in the lower limb with autologous BM-MSCs intramuscularly and significantly reduced the ulcer size (57,58). The clinical trial carried out by Hernández and coworkers on 22 patients with pressure ulcers due to spinal cord injury showed that injected autologous BM-MSCs topically had a better healing effect compared with traditional surgical treatment (59). Although MSCs show dramatic therapeutic effects on chronic wound healing, more attention should be paid to non-healing wounds that occur in ageing patients. As mentioned above, because donor age or culture senescence impacts the regenerative capability and differential potential of MSCs, it seriously limits the autologous application of MSCs in the ageing population. Therefore, it is imperative to highlight the impact of this relationship between MSC ageing and curative effects on chronic wound healing.

In recent studies, researchers demonstrated that aged adipose tissue derived MSCs are unable to rescue age-associated impairments in cutaneous wound healing because of their significantly compromised ability to support vascular network formation. Through single-cell transcriptional profile analysis, they found a sub-population of MSCs with depleted pro-vascular characteristics (60). Also, there is increasing evidence that aged cells lacked the anti-inflammatory, protective effect due to changes in the expression levels of inflammatory response genes, which indicate that MSCs undergo an age-related decline in their immunomodulatory activity (61). Because angiogenesis dysfunction and inflammation deletion mainly account for chronic wounds, there is no doubt that the therapeutic potential of MSCs in chronic wound healing will be hampered heavily by decreasing these capabilities.

Potential solutions for age-related impacts

Reduced oxygen concentration

A series of molecular and cellular changes occur in MSCs during in vitro culture (62). Treatment of MSC lines from donors of various ages with 5% oxygen environment permits the cells to grow more robustly and with less oxidative stress than the traditional 21% oxygen concentration (63). Although the MSCs are obtained from donors of different ages, they share similar cellular fitness, suggesting that low-oxygen concentration may neutralise the negative effects of age, effectively.

Lower temperature of culture

Cell culture in low temperature requires less oxygen consumption, such as a 10% reduction in hybridoma and baby hamster kidney (BHK) cells (64) which directly reduce the radical level produced by aerobic respiration (65) and decreased culture temperature can also alleviate stress-induced senescence and apoptosis levels (66). Similarly, in MSCs, culturing at a low temperature 32°C shows a significant decline in oxidative damage, radical production and induction of glutathione peroxidase activity by reducing oxidative stress. Culturing temperature also regulates stem cell capacity of self-renewal and maintains the multi-potency of MSCs by raising p53 and p21 levels to suppress differentiation (67,68).

Growth factor addition

MSCs gradually lose differentiation potential in culture as the result of culture stress or in vitro senescence. Some batches of foetal bovine serum markedly enhance culture stress or in vitro senescence of MSCs. In contrast, MSCs expanded with fibroblast growth factor-2 (FGF-2) maintain their trilineage differentiation potential at high levels throughout many mitotic divisions. Furthermore, FGF-2 markedly enhances MSC proliferation (69). FGF-2 may affect the innate properties of MSCs, but MSCs readily lose multi-potency in culture without FGF-2. Some other studies also used FGF-2 in MSC cultures (70).

Natural environment cultures are beneficial to maintain the properties of MSCs compared with traditional medium. Wharton's jelly extract (WJE) from UC-MSC niche, a commonly used supplement, which is abundant in collagen, fibronectin and insulin-like growth factor I (IGF-I) and basic fibroblast growth factor-b (bFGF) (71), is reported to preserve MSC properties effectively (72). The bottom of culture vessel coated with WJE facilitates in suppressing the MSC senescence through up-regulation of p53 and p16INK4a/pRb expression. Analysis at the molecular level shows decreased intracellular ROS in MSCs (73).

hTERT over-expression

Transfecting with human telomerase reverse transcriptase (hTERT) vector, the telomerase catalytic subunit, in vitro culture MSCs enhance genome stability by maintaining mitochondrial physiology to keep the oxygen consumption rate (OCR) and oxidative stress at a low level and normal antioxidant defences such as SOD2, which is significantly over-expressed in ageing cells. hTERT has also been demonstrated to be a transcriptional modulator that promotes metabolism and decreases ROS production (74). In addition, it markedly reduces aneuploidy level and prevents the dysregulation of ploidy-controlling genes through up-regulation of hTERT (75).

Nanog transfection

Pluripotency maintained transcription factor, Nanog (76), reverses the decline of proliferation and differentiation potential in BM-MSCs from adult donors through activation of the TGF-β pathway. The young MSCs express a high level of Nanog and microarray analysis results showed that adult BM-MSCs transfected Nanog close to the neonatal MSCs. It also made genes involved in the cell cycle, DNA replication and DNA damage repair up-regulated, which is definitely

facilitating the proliferation rate and clonogenic capacity. Notably, Nanog will be beneficial in treating cardiovascular diseases that are more likely to happen in elderly patients for its restoration of the myogenic differentiation potential and contractile function of BM-MSC (77). Besides, considering the safety factor, up-regulation of oncogenes may lead to cancer and man-made viral vectors may have potential to infect human cells. Therefore, it is essential to develop safer solutions for these obstacles.

Other approaches for compensation of MSC ageing

Although MSCs have demonstrated a reduced ability to improving chronic wound healing in case of ageing, there is still some compensation to ageing-induced impacts on MSCs. According to previous work, one of the underlying molecular mechanisms of mesenchymal stem cells facilitating chronic wound healing is through the paracrine factors to promote angiogenesis and improve impaired metabolism (78–80). Hence, we assume that up-regulation of the angiogenic factors and IGF-1 in the MSCs could neutralise the negative effects of ageing to some extent. On the other hand, beneficial niche for MSC regulation produced by tissue engineering approaches would be an effective strategy to remedy the potential limitation of MSC ageing. For instance, constructing microparticles with growth factors not only could enhance their interaction with MSCs by increasing its local concentration, but might also ameliorate the therapeutic effects on chronic wounds directly (81).

Summary and future prospective

Despite numerous advances in wound repair, some wounds never heal and become chronic problems that result in significant morbidity and mortality to the patient. Stem cell therapy for these chronic cutaneous wounds has recently emerged on investigation as a potential solution. Especially, MSCs are a promising source of adult progenitor cells as they are easy to isolate and expand and have been shown to differentiate into various cell lineages. However, along with ageing, MSCs are likely to suffer significant loss in number and functionality, resulting in progressive decline in tissue maintenance and regenerative capacity in the long-term (82). On the other hand, the prevalence of chronic wounds usually occurs in the ageing population. Thus, the ageing population has profound impact on MSC regenerative capacity and subsequently on its curative effects.

Age-associated changes in MSCs should be taken into account when they are intended for application in research or for cytotherapy for chronic wound healing. It is required to investigate the entire organism for the internal drivers and extracellular interactions at the molecular, cellular and organ levels, based on the substantial individual variation, with multiple experimental methods. It is also crucial for better approaches to isolate, expand and characterise MSC populations. In addition, resolutions of ageing-related stem cell changes are required to identify specific pathways involved in the activation of MSCs, which is important in the regeneration of a complete and functional tissue.

Future directions for research in this field might focus on optimization of MSC efficiency in the chronic wound context, both by improving cell function via independent technologies or in combination with tissue engineering designs as well as niche regulation. Moreover, ongoing promising strategies to extend MSC survival and optimise cell delivery continue to emerge, which will improve the regenerative potential of these ageing MSCs in the future.

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