Bioactive Materials 6 (2021) 666–683

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/biomat

Advancing application of mesenchymal stem cell-based bone tissue regeneration

Fengqing Shang[a,b,1], Yang Yu[c,1], Shiyu Liu[a,1], Leiguo Ming[a], Yongjie Zhang[a], Zhifei Zhou[d], Jiayu Zhao[e], Yan Jin[a,*]

[a] State Key Laboratory of Military Stomatology & National Clinical Research, Center for Oral Diseases & Shaanxi Key Laboratory of Oral Diseases, Center for Tissue Engineering, Fourth Military Medical University, Xi'an, Shaanxi, 710032, China
[b] Department of Stomatology, The 306th Hospital of PLA, Beijing, 100101, China
[c] Department of Periodontology, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University & Shandong Key Laboratory of Oral Tissue Regeneration & Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, Jinan, Shandong, 250012, China
[d] Department of Stomatology, General Hospital of Tibetan Military Command, Lhasa, 850000, China
[e] Bureau of Service for Veteran Cadres of PLA in Beijing, Beijing, 100001, China

ARTICLE INFO

Keywords:
Mesenchymal stem cells
Bone healing
Regenerative medicine
Biomaterials
Extracellular matrix
Cytotherapy
Cell-sheets/ -aggregates
Exosomes

ABSTRACT

Reconstruction of bone defects, especially the critical-sized defects, with mechanical integrity to the skeleton is important for a patient's rehabilitation, however, it still remains challenge. Utilizing biomaterials of human origin bone tissue for therapeutic purposes has provided a facilitated approach that closely mimics the critical aspects of natural bone tissue with regard to its properties. However, not only efficacious and safe but also cost-effective and convenient are important for regenerative biomaterials to achieve clinical translation and commercial success. Advances in our understanding of regenerative biomaterials and their roles in new bone formation potentially opened a new frontier in the fast-growing field of regenerative medicine. Taking inspiration from the role and multicomponent construction of native extracellular matrix (ECM) for cell accommodation, the ECM-mimicking biomaterials and the naturally decellularized ECM scaffolds were used to create new tissues for bone restoration. On the other hand, with the going deep in understanding of mesenchymal stem cells (MSCs), they have shown great promise to jumpstart and facilitate bone healing even in diseased microenvironments with pharmacology-based endogenous MSCs rescue/mobilization, systemic/local infusion of MSCs for cytotherapy, biomaterials-based approaches, cell-sheets/-aggregates technology and usage of subcellular vesicles of MSCs to achieve scaffolds-free or cell-free delivery system, all of them have been shown can improve MSCs-mediated regeneration in preclinical studies and several clinical trials. Here, following an overview discussed autogenous/allogenic and ECM-based bone biomaterials for reconstructive surgery and applications of MSCs-mediated bone healing and tissue engineering to further offer principles and effective strategies to optimize MSCs-based bone regeneration.

1. Introduction

The demand for tissue engineered bone is huge due to the high incidence of large segmental bone defects, resulting from trauma, inflammation, or tumors [1]. However, the human body has a limited ability to correctly auto-regenerate most, if not all, of its major tissues and organs when the original tissue integrity has been seriously damaged as a result of medical disorders involving tissue dysfunction or devastating deficits [2,3]. Specifically, reconstruction of bone defects with mechanical integrity to the original surrounding bone tissues is important for patients' rehabilitation [4]. Thus, autogenous bone tissues are the most commonly used graft material for its osteogenic potential [5,6]. Not only autografts, but allografts have been used to treat bone defects, in which, the autologous bone grafts are regarded as the gold standard for many indications, however, there are still many limitations [7]. In the process of the peruse for the substitutes materials, various artificial bone grafts made of metal alloys, titanium mesh, ceramics, porous hydroxyapatite material, or synthetic polymers, were

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author.
E-mail address: yanjin@fmmu.edu.cn (Y. Jin).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.bioactmat.2020.08.014
Received 18 April 2020; Received in revised form 7 August 2020; Accepted 15 August 2020

previously reported to be used in endogenous bone healing, however, their effects were not hopeful. For instance, the insertion of artificial materials such as metal alloys require the removal of a significant amount of adjacent bone; inherently lacks of native growth factors lead to an absence of osteoinductive properties; problems can arise at the prosthetic material/bone interface and give rise to a clinical immunogenic response and so on [8–13]. Although these drawbacks are now advanced by the use of a natural ECM toward fabricating bone substitute materials, many of these substitute material tissues fail to fully match the functional properties of native bone tissues [14–16].

Regenerative medicine is defined as regrowth of lost or destroyed parts of tissues or organs [17]. So, when faced with an ever-increasing incidence of critical sized trauma, degenerative diseases and metabolic disorders, and so on, regenerative medicine and tissue engineering promise to develop new biological therapeutics to treat a diverse range of diseases that are currently intractable and are alternative therapeutic strategies which could facilitate bone regeneration. Tissue engineering is an interdisciplinary field that connects various scientific aspects from engineering, materials science, biology, and medicine, thus developing a novel bone transplanting system including suitable scaffold materials and feasible seed cells play critical role for basic research and clinical work in the field of bone regeneration [18]. Thanks to great advancements in stem cell biology, new therapeutic strategies have been made possible with the aim of regenerating tissues injured by a number of diseases [19,20]. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive and can give rise to progeny that differentiate into any of the specialized cells of embryonic or adult tissue (Stedman's Medical Dictionary, 2002). Stem cells constitutes a heterogeneous population of cells and there are two main types of stem cells, embryonic and non-embryonic. Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst and can differentiate into cells of all three germ layers of the mammalian body including the germ cells [20,21]. Other non-embryonic stem cells, mostly adult stem cells, are already somewhat specialized and have limited differentiation potential as shown in Table 1 [20,22]. Among these stem cells, MSCs, one type of adult stem cells, are generated through enforced expression of defined transcription factors, which reset the fate of somatic cells to an embryonic stem-cell-like state [20]. These stem cells can be found in a number of adult tissues including adipose tissues [23], bone marrow [24,25], peripheral blood [26,27], skin, pancreas, intestine, brain and hair follicles, as well as in the teeth [28,29]. Unlike ESCs, which are more controversial in ethical problem, MSCs are considered to be a more appropriate cell source for bone tissue engineering [30]. On the other hand, in the process of pursuing a perfect tissue engineered template, it is also important to consider whether we have looked too far ahead and missed the readily available building blocks, such as naturally derived biomacromolecules required to create biomaterials for this purpose [31]. Thus, biomaterials scientists aim to recreate the intrinsic properties of human-derived biomaterials as regenerative biomaterials tailored to specific applications. Among these, the use of ECM components and technique of cell-sheets and cell-aggregates engineering would mostly reserve the ECM, which could be considered as a scaffold tissue to be used as transplantable tissue [32,33]. Meanwhile, as a class of feasible seed cells, MSCs based cell engineering transplanting was more and more used in recent years [34–38]. So far, cell-sheets and cell-aggregates based tissue engineering technology has already been applied for the treatment of several kinds of tissue defects such as cornea, myocardium, periodontal ligament and bladder, as well as bone tissues [24,25,39–42].

However, it is still an unfulfilled challenge to restore extensive bone loss and defects in the regard of bone metabolism-related diseases, resulting in a notable incidence of morbidity particularly in elderly people [43,44]. For instance, osteoporosis, which is a common and preventable disorder, predisposes individuals to an increased risk of suffering from bone fracture. Specifically, further complicating matters, non-healing bone often occurs with comorbidities, such as diabetic or postmenopausal bone fractures and osteoporosis induced by aging and diseases, which provide more difficulties in bone recovery [45,46]. Pathologically, in extensive bone loss and impaired bone healing, MSCs suffer from functional decline including diminished viability and disordered differentiation, which could be attributed to at least 3 major microenvironmental alterations of hormonal, inflammatory and metabolic conditions [47,48]. Although MSCs-mediated bone healing has shown great promise, their efficacy was not always achieved, exerting conditional benefits in diseased microenvironments [49–52]. As the microenvironmental challenges to MSCs-based bone regeneration become emergently important, application solutions have been accordingly developed, including but not restricted to recreating beneficial microenvironment to guarantee MSCs-based regeneration, improving resistance of MSCs to diseased microenvironment, and usage of subcellular components of MSCs [51,53,54]. Thus, establishing novel approaches to optimize MSCs-based bone regeneration in diseased situations is therefore in an urgent need to promote clinical therapeutics of bone healing. Following an overview of the characteristics and function of MSCs during bone tissue regeneration and bone grafts including auto-/allo-transplantation as original practice in this field, this review will discuss naturally derived bone graft substitutes for bone regeneration and will highlight MSCs based strategies, even though these approaches are in their early stages of development. The potential challenges and obstacles faced with bone healing and the viable and effective strategies to optimize MSCs-based bone regenerative therapies in pathophysiological conditions are also discussed.

2. The characteristics of bone metabolism and regeneration

Bone is a highly dynamic tissue in the body that remodels and regenerates itself throughout adult life. Besides, it is a complex organ with numerous functions including hematopoiesis, regulation and storage of key minerals, the protection of vital life-sustaining organs, facilitation of locomotion, etc. Pathologically, bone remodeling process has been impaired. A clear understanding of the principles underlying bone loss and repair is essential for the treatment of traumatic injuries (fractures and non-unions), patients with bone infection, osteonecrosis, arthritis,

Table 1
Normal differentiation pathways of adult stem cells from various tissues and cells [22].

Stem cell	Source	Type of cells produced
Hematopoietic	All types of blood cells	Red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, macrophages and platelets.
Bone marrow Stromal cells (mesenchymal)	Connective tissues	Tendons, osteocytes (bone cells), adipocytes (fat cells), chondrocytes (cartilage cells)
Neural	Parts of the nervous system	Neurons, astrocytes and oligodendrocytes
Epithelial	Lining of the digestive tract	Absorptive cells, goblet cells, paneth cells and endocrine cells
Epidermal	Basal layer of epidermis	Keratinocytes and dermal cells
Follicular	Base of hair follicles	Hair follicles and epidermis
Hepatic	Liver	Hepatocyte cells

osteoporosis, spinal fusion, wear particle associated osteolysis, metabolic bone disease, tumors and other diseases affecting bone [55]. Thus, the subject of bone loss and subsequent repair has great clinical and economic importance. Recently, the recognition of MSCs contributions and the crosstalk between them and immune system have enabled us to gain a better understanding how these differentiation processes occur in physiological and pathological conditions and enlighten the potential therapeutics with coexistence of promising approaches and application challenges.

2.1. Mesenchymal origin cells participation in bone remodeling

Bone remodeling is a lifelong process in which mature bone tissue is removed from the skeleton by bone resorption and is replenished by new during ossification or bone formation, which could maintain the stable bone mass during adult life and following rapid skeletal development and growth [56,57]. During these process, bone structural integrity requires precise regulation involving the osteoblast, a cell claimed to be of mesenchymal origin, which orchestrates bone formation, the osteoclast, a cell of hematopoietic origin that orchestrates bone resorption, and osteocyte, a cell of osteoblasts origin during the final phase of bone remodeling [58–60]. Among these cells, osteoblasts and osteoclasts with coupled but opposing actions are thought to be commonly influenced by MSCs in the cellular level, as MSCs are the precursors of osteoblasts and regulators of osteoclasts [57,61,62].

In addition to this process of remodeling, bone has a remarkable potential for regeneration, during which the MSCs have the most prominent function. The discovery of the MSCs are dated back to Alexander Friedenstein and coworkers, demonstrating that a subpopulation of non-hematopoietic stromal cells exists in postnatal mammalian bone marrow stroma and were defined as to meet the criteria established by the International Society of Cellular Therapy [63]. These criteria include an ability to adhere to plastic, the expression of a number of cell markers, including CD105, CD73, and CD90 while undergoing differentiating into a full range of functional skeletal tissue cells (bone, cartilage, adipose tissue, and myelosupportive stroma) under appropriate experimental conditions, and the ability to self-renewing [64,65]. Indeed, MSCs are of particular interest as they have these capacity described above and thereby maintain the homeostasis and repair of bone tissue, which is continuously being remodeled throughout adulthood and undergoes repairs during fracture healing.

2.2. The role alterations of MSCs and immunity of bone tissue in disease

MSCs clearly have prominent role during the process of bone metabolism in diseased conditions whether direct or indirect, as they act as a source of progenitors for osteoblasts and osteoclast. When bone defects or fracture happened, the endogenous MSCs could migrate to the damaged site and participate the bone tissue reconstruction. Currently, the putative contributions to skeletal homeostasis have made MSCs the focus of extensive investigations worldwide to elucidate pathogenesis of bone diseases [66,67]. Notably, MSCs derived from inflammatory microenvironments demonstrated an inhibition in bone regenerative potential, underlying impaired bone healing mediated by autologous MSCs [53,68] (Fig. 1). Our team found that MSCs from estrogen deficiency osteoporosis rat showed a delayed healing of bone fracture [69] and exhibited decreased osteogenic differentiation potential compared with normal MSCs [70]. More specifically, MSCs exhibited impaired capability of inducing apoptosis of osteoclasts in estrogen deficiency, contributing to elevated bone resorption rates in bone loss [71]. In osteoporosis of diverse pathologies, MSCs from bone marrow, BMMSCs genera, also demonstrated a differentiation switch from osteogenesis to adipogenesis with a frequent decline of self-renewal capacity, underlying decreased bone formation rates with increase marrow adiposity [72,73]. Besides, bone marrow mesenchymal stem cells (BMMSCs) also showed more intriguing behavioral changes and general functional alterations reflecting the respective pathogenesis [74].

Despite the heterogeneity of MSCs disorders in detailed bone pathologies, tissues have evolved a number of strategies to preserve their precious pools of stem cells in the face of harm. When bone is subjected to injuries, pro-inflammatory stimuli (trauma, infection and so forth), the biological processes regulated by the innate immune system ensue, as with other tissues and organ systems, to affect local repair and bone healing. Restoring tissue integrity during and after an active immune response is paramount for limiting pathology and promoting inflammatory resolution. Indeed, the dialog between stem cells and immune cells in wound repair and in inflammation has ancient roots. Thus, it is reasonable to expect that macrophages and regulatory T cells (T_{regs}) serve the dual functions of modulating immunity and promoting regeneration: they seem to either repurpose their existing immune pathways and/or adopt novel pro-regenerative functions to engage stem cells and short-lived progenitors [75]. As a known reservoir for T_{regs}, the inflammatory stress of bone marrow were limited through T_{regs} producing the immune suppressive cytokine interleukin-10 (IL-10) in the vicinity of hematopoietic stem cells (HSCs) [76]. In the absence of bone marrow T_{regs}, HSCs were fewer in numbers and displayed increased sensitivity to oxidative stress [77]. Given their burgeoning role in controlling stem cells, conversations between stem cells and tissue-resident immune cells seem likely to become unhinged in disease states [78–81]. The stem cell-immune cell interactions include not only macrophages and T_{regs}, but also other tissue resident immune cells, such as innate lymphoid cells. Multiple subsets of innate lymphoid cells can play context-specific roles in directing regeneration and differentiation of stem cells [82,83]. In the bone marrow where these immune effectors are generated, neutrophils modulate the HSCs niche and exert their influence on HSCs retention and regeneration [84,85].

3. Tissue graft biomaterials for bone tissue repairing

The science of reconstructive surgery has experienced a remarkable advance, as well as grafts safety and feasibility have largely been improved in surgical technology, thus not only soft- but hard-tissue grafts have grown in popularity for tissue reconstruction in routine clinics. However, alongside the shortage of human origin tissue grafts available for use in patients, have prompted the use of natural biomaterials, which innate ability could promote biological recognition and may positively support cell adhesion and function [31]. Natural biomaterials present a crucial subset of biomaterials for use due to their bioactivity, biocompatibility, tunable degradation and mechanical kinetics and their intrinsic structural resemblance of native tissue ECM. Understanding these native bone grafts tissue composition and structure and revisiting the observed clinical benefits of these tissue grafts can offer practicing tissue engineers important information on state-of-the-art biomaterial evolution and design inspiration (Fig. 2).

3.1. Bone grafts of human origin

The use of biomaterials of human origin for therapeutics was first rooted in tissue grafts transferred from one site to another site within the same individual. Tissue grafts (autogenous or allogenic tissue grafts) of human origin derived from bone have a long and successful history of use as “living tissue replacements” to treat selected conditions, such as small- or medium-sized bone defects [86]. The discuss of advantages and disadvantages of human origin bone grafts will provide surgeons validated principles regarding bone grafts choice during clinical practice.

3.1.1. Autologous bone grafts

Many clinicians consider harvested autologous tissue to be the best material for the reconstruction of most, if not all, tissue defects as the ultimate goal for tissue engineering strategies is to develop a tissue

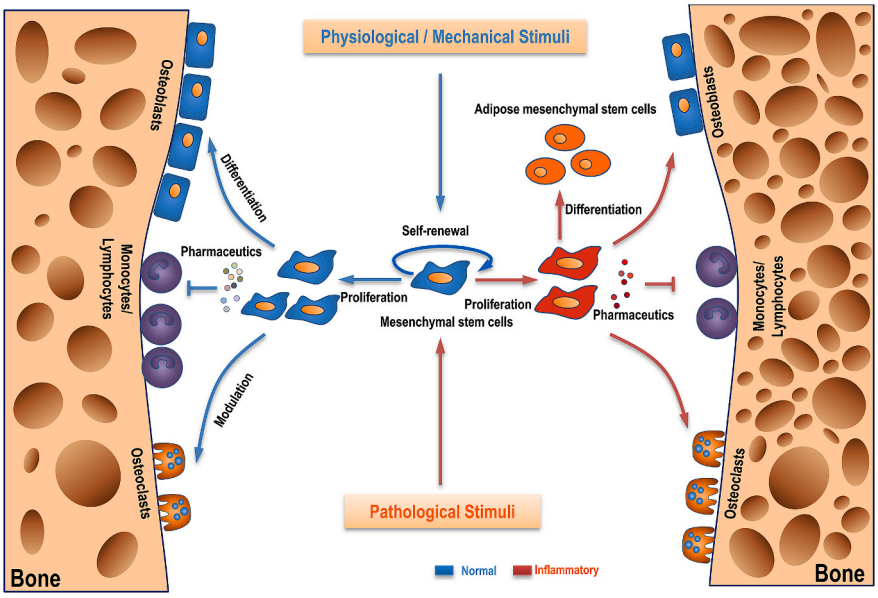

Fig. 1. MSCs contributions to bone remodeling and pathology.

construct that has biological performance identical or similar to that of an autologous tissue graft upon implantation. Even today, autologous tissue grafts, also called autografts, are still the gold standard with which all other implantable biomaterials are compared as bone autografts from a patient's own body deliver no risk of immunological rejection; possess complete histocompatibility; and offer superior osteogenic, osteoconductive and osteoinductive performance (Fig. 2A) [87]. The transplantation of a fresh bone autografts are an attempt to achieve rapid bone restoration because living bone can survive well and add to bone volume at a recipient site and eventually maintain bone strength. Historically, cancellous bone/bone marrow autografts have been proved as the best osteoinductive bone grafting, as it can offer a rich source of bone and marrow cells that have osteogenic potential (Fig. 2B) [88]. Moreover, the vascular response in autologous cancellous grafts is much greater than that in cortical autografts, as the existence of native spaces within their structure permits the diffusion of nutrients necessary for new bone formation and allows limited revascularization through the microanastomosis of circulating vessels [89–91]. While, in the regard for the treatment of bone defects requiring immediate structural support, cortical bone autografts are good

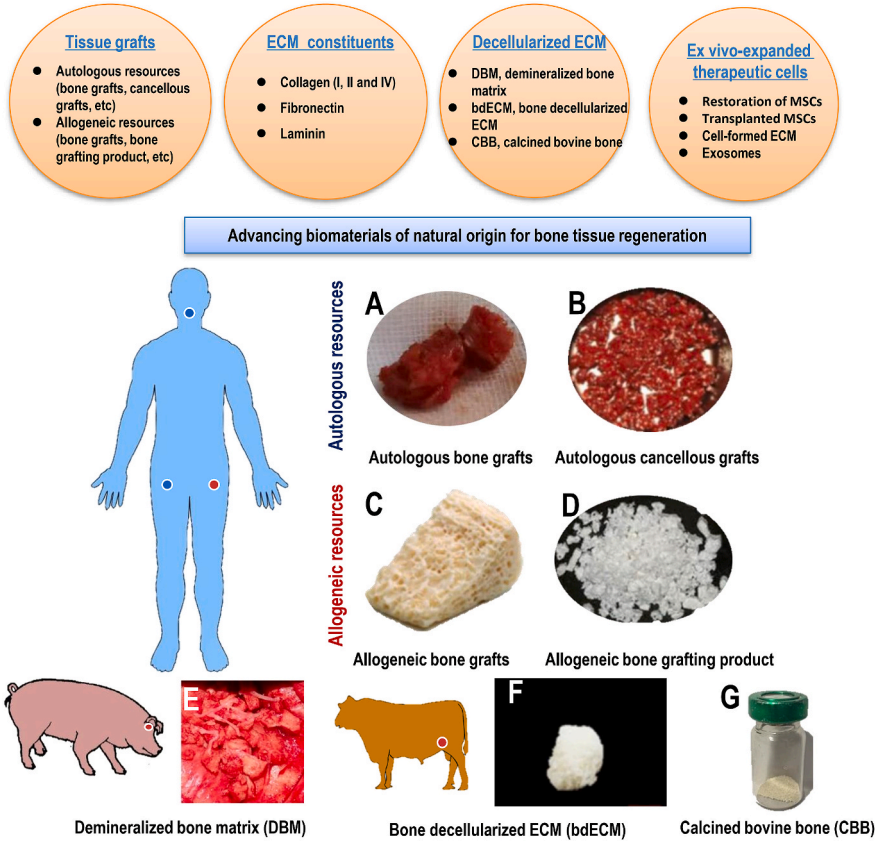

Fig. 2. Schematic representation of bone tissue grafts of natural origin bone graft substitutes for pre/clinical therapeutics (examples are given where appropriate; illustrations are Not to scale). Bone tissues for transplantation include autologous bone grafts (A), autologous cancellous bone grafts (B), allogeneic bone allografts (C), allogeneic bone grafting materials produced product (D), demineralized bone matrix (DBM) (E), bone decellularized ECM (bdECM) (F) and calcined bovine bone (CBB) (G). The images used here are selected samples for schematic representation only; they do not represent any particular preference by pre/clinicians. Source: Figure components B, C, D and F reproduced with permission from the authors; the remaining components are by the corresponding author's, or from unpublished resources from the corresponding author's institution.

choices. Although cortical grafts may not be revascularized as rapidly or properly as cancellous bone graft did and it is osteoconductive but lack osteoinductive properties; the surviving osteoblasts within the transferred bone do provide certain osteogenic properties [92,93]. Thus, autologous bone grafts are the predominantly considered osteoconductive materials for bone replacement, with success rates of well over 90% [94]. Multiple clinical concerns, however, have largely limited the transfer of autografts, including the possibility of surgical complications and pain associated with the donor site, amount of available graft material, short-term viability and unpredictable graft resorption [90,95].

3.1.2. Allogenic bone grafts

Compared to expense and trauma associated with autograft harvesting, the quantity and size limitations of grafts and donor site morbidity are no longer concerns in the allogenic bone grafts (Fig. 2C). The osteogenic properties of allografts are weak as the absence of living cells, but these are still in possession of both osteoconductive and weakly osteoinductive abilities upon implantation because they release bone morphogenetic proteins (BMPs) that coax bone-forming cells [96]. In brief, this type of grafting material is attractive as it closely matches the recipient in constitutional elements and architecture and is theoretically available in unlimited quantities (Fig. 2D). The fundamental problems of this grafting material are antigenicity and the potential for infectious agent transmission, which is a major consideration that is in fact minimized by recent strategies associated with tissue processing including sterilization and freezing. However, these procedures in turn decrease graft properties with regard to osteoinduction, osteoconduction and mechanical strength, and indeed, real and perceived risks of disease transmission still exist [97,98].

3.2. ECM based bone graft substitutes

Tissue engineering emerged over several decades, several of the principles established by those pioneering explorers are still followed by the scientists working in current bioengineering disciplines; be it tissue (or organ) auto-/allo-transplantation or tissue engineering (or regenerative medicine), the essential goals remain the same. Scientists have learned that the human body is a tremendous potential source of bio-scaffolds and biopolymers for therapeutics; these biomaterials have attracted considerable attention in the tissue engineering and regenerative medicine communities [99,100]. Biomaterials play a pivotal role in the success of tissue engineering, so to either create living neotissues in vitro that are similar or identical to their native body counterparts or temporary biodegradable support matrices with natural, tissue-resembling structural and functional attributes are generally necessary, if not indispensable, for cell attachment and housing [101–105]. Therefore, the philosophy of the emerging discipline of current tissue engineering and, indeed, of regenerative medicine is focusing on creating ECM-mimicking biomaterials or modulating stem cell niches that recapitulate pivotal interactions with host cells to unlock the patient's own regenerative ability for organization and self-repair [105–107]. ECM is synthesized and assembled by the resident cells; has a highly complex regulated, tissue specific composition and set of physical properties in the majority of tissues and organs in the human body; and the nature of its contact with stem cells also varies considerably [106]. Thus, ECM-based biomaterials that should retain the native materials and proteins of a living tissue/organ, along with the innate spatial arrangements in certain cases, provide appealing tissue engineering templates on which either exogenously transplanted or endogenously recruited cells may adhere, proliferate, differentiate and ultimately integrate to form functional tissues [108–110].

The use of ECM assemblies directly or indirectly derived from human tissues/organs for tissue engineering applications opens a new route for scaffolding biomaterial design. Considerable efforts have been made to synthesize biomaterials based on the functionality of natural ECM molecules to guide morphogenesis in tissue engineering and regenerative medicine [111]. A variety of bone substitute materials whether degradable or not could introduce bone tissue regeneration by providing three-dimensional (3D) scaffold structure in local microenvironment. In a recent study, non-collagen proteins (NCPs) from bone ECM combined with 3D nanofibrous gelatin (NF-gelatin) scaffolds were used to form a material device mimicking both the chemical composition and the nanostructured architecture of natural bone ECM. The incorporation of NCPs into the surfaces of the device was found to result in significant osteogenesis and mineralization, leading to new bone regeneration, suggesting that biomimics are a new signpost for future cell scaffolds and tissue engineering templates [112]. Moreover, the composite of the a phosphorylated polymer, poly (sebacoyl diglyceride) phosphate, named as PSeD scaffold, and adipose-derived mesenchymal stem cells (ADMSCs) derived ECM— ADM can be used to mimic a biophysical microenvironment and synergistically stimulated bone formation in vitro and in vivo through facilitating the adhesion and growth of BMMSCs and providing a suitable microenvironment for osteogenic differentiation [113,114].

Decellularized tissue-derived biomaterials, which are created by the elimination of all cellular and nuclear materials mineralized bone allografts can be removed inorganic elements, yielding a natural polymer product that is generally referred to as demineralized bone matrix (DBM) in bone tissue (Fig. 2E) [115,116]. As an acellular organic matrix, DBM-derived from the native tissue mimics the microstructure and major protein components of native bone but is less immunogenic and possesses good osteoinductive and osteoconductive performance [117,118]. Through an interdisciplinary endeavors by biologists, materials scientists, engineers and physicians, tissue-engineered products for bone have been approved for clinical application by the United States FDA. A number of ECM products based on bone tissues are already commercially available (Table 2) [119]. DBM materials have shown an advanced effect in healing the bone defects than the synthetic materials, however, there are still several concerns related to the safety and efficiency [120,121]. In the clinic application, it has been found that DBM cannot provide structural support so its primary application is in structurally stable places such as the sites of bone defects. When applied, DBM can be used in association with a cancellous bone autograft when the defects is very large and the supply of autologous bone is insufficient. Some issues may also be involved the manufacturing process, sterilization and storage methods of DBM and the osteoinductive properties can be change from donor to donor [122]. Specifically, the fundamental structure and entrapped growth factors in ECM may be insufficient exposed and cannot be released but be obtained by hydrogen chloride (HCl) solution during the process to remove residual inorganic component of raw DBM materials, which can effectively promote the bone healing; the effect of the DBM commercialized or used in a clinical field in the bone healing may not be maximized as they does not contain ECM-like microstructure and cannot release the growth factor remaining inside of the materials; and residual cellular component in the DBM can cause unexpected adverse effects, which may be involved in animal deaths or severe inflammatory reaction in vivo [123].

Herein, a native bone decellularized ECM (bdECM) from bovine bone were extracted via demineralization and decellularization processes to remove inorganic components and to exposure ECM structure while maintaining higher concentrated amounts of bone-forming bioactive molecules such as bone morphogenetic protein-2 (BMP-2) and bone morphogenetic protein-7 (BMP-7). Furthermore, contamination by residual DNA was significantly reduced to meet the minimum standards of immune response during the fabrication process which ensure the safety issue different from the DBM in medical applications. These features enabled bdECM successfully promote mineralization of the cultured primary osteoblasts in vitro and improve mature bone regeneration without any further inflammatory reaction, which were significantly better than those from DBM (Fig. 2F) [123]. Another

Table 2
Partial list of commercially available extracellular matrix (ECM)-based products of human origin for bone tissue reinforcement or replacement.[a] [119].

Product	Company	ECM source	Application focus	Product form (brief description)
Puros® DBM	Zimmer	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency)	Allograft demineralized bone matrix (DBM) putty (a natural polymer product produced by a mineralized bone allograft following the removal of its inorganic elements using a demineralizing agent; 100% derived from allograft tissue)
AlloMatrix®	Wright Medical Technology Inc.	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency, augmentation in situations of segmental bone loss and interbody spinal fusion)	Allograft demineralized bone matrix (DBM) putty (a natural polymer product produced by a mineralized bone allograft following the removal of its inorganic elements using a demineralizing agent; 100% derived from allograft tissue)
AlloFuse®	AlloSource	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency)	Injectable gel or putty (DBM combined with heat-sensitive copolymer)
InterGro®	Biomet Osteobiologics	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency and segmental bone loss)	Paste, putty or mix containing hydroxyapatite/calcium carbonate composite granules (DBM in a lecithin carrier)
Grafton®	Osteotech Inc.	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency)	Gel (DBM combined with glycerol)
Osteofil®/Regenafil®	Regeneration Technologies	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency, augmentation in situations of segmental bone loss and interbody spinal fusion)	Injectable paste or putty, strips and blocks with cortical cancellous chips (CCC) (DBM combined with a non-toxic natural gelatin carrier)
Optefil®	Exactech Inc.	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency and segmental bone loss)	Injectable bone paste/dry powder ready to be hydrated (DBM suspended in a gelatin carrier)
Opteform®	Exactech Inc.	Bone	Bone repair (e.g., reconstruction of cavitary bone deficiency and segmental bone loss)	Formable putty or dry powder ready to be hydrated (DBM and CCC suspended in a gelatin carrier)
Optecure®	Exactech Inc.	Bone	Bone repair (e.g., reconstruction and augmentation of deficient maxillary and mandibular alveolar ridges and dental intraosseous defects)	Dry mix delivered with buffer solution (an optimal concentration of DBM suspended in a resorbable hydrogel carrier)
Optecure® + CCC	Exactech Inc.	Bone	Bone repair (e.g., reconstruction of the spine, pelvis and extremities)	3D matrix delivered with buffer solution (DBM and CCC suspended in a hydrogel carrier)

[a] The product list does not represent any particular preference by the authors.

calcined bovine bone (CBB), came from the high-temperature calcinated femur of healthy cattles and were broken into particles with different diameters has been confirmed that CBB can provide microenvironment for osteogenic and for BMMSCs to survive, grow and differentiate no matter in vitro or in vivo by means of its porous characteristic (Fig. 2G) [25]. This material also has a good strength and biocompatibility.

4. Potential therapeutics of MSCs in bone regeneration

Although research advances in bone biofabrication and bioactive materials, the approach that is most likely to make a real difference in transplantation in the long term is regenerative medicine. With the extension of the concept of biomaterials, that can now include many substances, such as engineered constructs as described above, as well as therapeutic cells, that may generally not be considered as biomaterials in the past [103,104]. As a new concept in using biomaterials of natural origin, MSCs have been used in regenerative procedures, because they may participate in the wound healing cascade during therapeutic regeneration. Until 2015, more than 500 clinical trials of MSCs therapy are approved worldwide [124].

4.1. MSCs based cytotherapy

The term cell therapy is prescribed by the American Society of Gene & Cell Therapy (ASGCT) as “the administration of live whole cells or maturation of a specific cell population in a patient for the treatment of a disease” [125]. MSCs are becoming promising candidates in cell-based therapeutics for an unexpectedly wide variety of human diseases, including those involving autoimmunity, inflammation and regenerative medicine, due to the immunomodulatory, anti-inflammatory and the multi-differentiation properties [126,127]. In addition to its fundamental science, researchers hold high promise for MSCs cell therapy partly because MSCs are considered as “safe cells”, which suggest the great therapeutic potential of MSCs for bone or other tissue repair and regeneration [128]. Concerted efforts have been and are still being made in the identification and delivery of ex vivo-expanded MSCs-mediated bone healing has shown great potential, however, these cell populations’ economic and clinical feasibility continues to present formidable challenges especially in diseased microenvironments (Fig. 3 &Table 3) [49–52].

4.1.1. Restoration of endogenous MSCs function

A strategy to facilitate bone healing is that the deficient resident MSCs can be supplemented via a mobilization of endogenous MSCs from other sites to rescue the MSCs deficiency in systemic and local bone loss. To accelerating the migration of endogenous MSCs, LLP2A-Ale and erythropoietin, as migration stimulators, have been proved can direct MSCs to bone formation surfaces and could accelerate bone formation in both fracture sites and even osteoporosis induced by aging and estrogen deficiency as shown in Table 4 [129–131]. In addition, biomaterials with bioactivity have been developed, with the potential to create local beneficial microenvironments for directing regeneration of endogenous MSCs. It has been reported that scaffolds releasing related agents protein, including stromal cell-derived factor-1 (SDF-1), transforming growth factor β3 (TGF β3) and, connective tissue growth factor (CTGF) have been developed to enhance osteochondral regeneration and to promote bone regeneration in defects models through directing endogenous MSCs homing [132–134]. Furthermore, preferential niche for endogenous MSCs-based osteoporotic fracture healing can be achieved via application of bioactive agents, such as icariin loaded on calcium phosphate cement scaffolds to promote osteogenesis and angiogenesis of endogenous MSCs in ovariectomized (OVX) calvarial defects [135].

In bone fracture healing, inflammation is thought to be an essential process that precedes bone formation and remodeling [136]. Indeed,

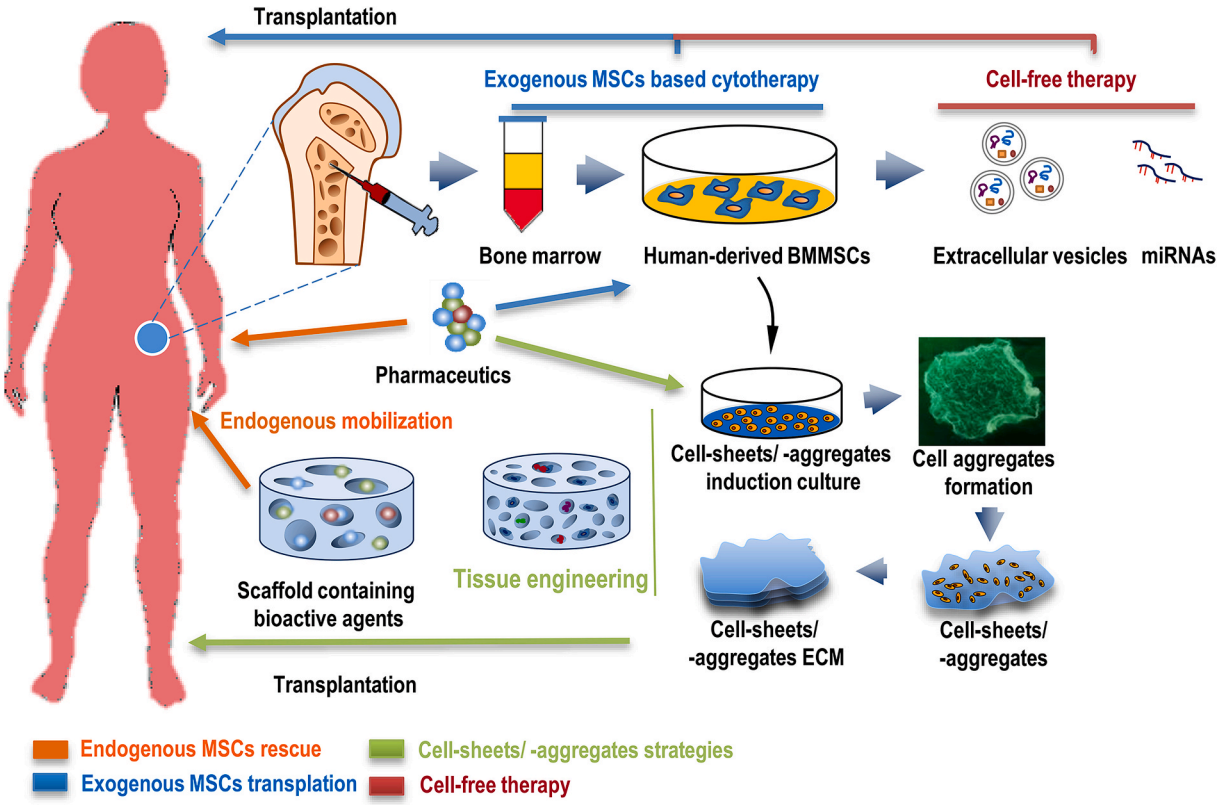

Fig. 3. Schematic of human-derived mesenchymal stem cells for cytotherapy and tissue engineering applications (Taking bone marrow mesenchymal stem cells for example, schematic is not to scale).

pro-inflammatory cytokines impairs resident MSCs not only in inflammatory and autoimmune diseases [137], but also in aging and estrogen-deficient conditions as the pivotal secondary detrimental factors [138,139]. The findings on diseased microenvironmental impacts on MSCs have prompted further establishment of rescuing approaches to endogenous MSCs disorders. On one hand, given the functional alterations of endogenous MSCs in diseased situation, multiple intervention targets in mediating MSCs disorders within a signaling network have also been examined in preclinical studies as shown in Fig. 4 [48]. At present, gene expression regulators including rapamycin, the mTOR signaling inhibitor [73,137]; DAPT, the Notch signaling inhibitor [53]; PDTC, the nuclear transcription factor-kappaB (NF-κB) signaling inhibitor [140]; GSK2606414, the endoplasmic reticulum stress inhibitor [141]; NAC, the antioxidant NAC [142]; and licochalcone A, the small molecular compound [143] have been proved as the promising effective agents in vivo. Furthermore, epigenetic modulators such as the histone methylation regulators DZNep [144], pargyline [145] and JIB-04 [146], as well as microRNAs (miRNAs) regulation based on adenovirus [68] liposomes [147] and scaffolds [148–150], have been successfully used to rescue endogenous MSC defects and promote bone regeneration in osteoporosis and fracture healing. Alternatively, therapeutics via pre-normalizing recipient microenvironmental has shown potential to rejuvenate endogenous MSCs for bone healing. In this regard, anti-inflammatory therapy has emerged as an important approach to restore endogenous MSCs function thus curing bone diseases. Several critical inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) have been elucidated to synergistically induce impairments of osteogenesis of BMMSCs in osteoporosis, via concerted mechanisms of signaling pathways and epigenetic modulations [139,151]. Thus, a classical anti-inflammatory drug, aspirin, has been tested that systemic application of aspirin via oral feeding significantly reduces the levels of TNF-α and IFN-γ, resulting in blockage of MSC deficiency and OVX-induced bone loss [52,139,152]. Based on the finding that, anti-TNF-α and anti–IFN–γ treatments could

Table 3
Challenges of MSCs-based bone regeneration in bone loss/defects.

MSCs rescue/mobilization	MSCs infusion in cytotherapy	MSCs transplantation in tissue engineering	Cell-free strategies
Current strategies			
1.Migration stimulators 2. Functional regulators 3.Scaffold-based delivery	1. Systemic MSCs infusion 2. Infusion of MSCs with genetic modifications	Cell-sheet/cell-aggregate technique	1. MSC-derived extracellular vesicles (exosomes) 2. miRNAs
Challenges			
1. Benefits not persistent 2. Potential side effects 3. Not etiological rescue	1. Use of autologous MSCs 2. Donor and cultural impacts on MSC function 3. Long-term survival of transplanted MSCs In 4. Recipient comorbidities 5. Recipient control of regenerative efficacy		1.Low yielding 2.Unreliable identified and purified method 3. Unprofiled and undetermined exosomal contents and function 4. Undetermined function of miRNAs

Table 4
Strategies enhance MSCs-based bone regeneration.

Properties	category	Bioactive molecules	Effect	Studies
Cell-targeted strategies				
Migration stimulators	Pharmaceutics	LLP2A-Ale,	Accelerating the migration of endogenous MSCs and directing MSCs	[129,130]
	Glycoprotein hormone	Erythropoietin	to bone formation surfaces	[131]
	Growth factors	SDF-1	Released by scaffolds to direct endogenous MSCs homing	[132]
		TGFβ3		[133]
		CTGF		[134]
Signaling pathway regulators	Pharmaceutics	Rapamycin	mTOR signaling inhibitor	[73,137]
		Licochalcone A	Up-regulating FasL in MSCs	[24,143]
		Osthole	Improving the capacity of osteogenic differentiation of MSCs	[240,241]
	Signaling inhibitor	DAPT	Notch signaling inhibitor	[53]
		PDTC	NF-κB signaling inhibitor	[140]
		GSK2606414,	Endoplasmic reticulum stress inhibitor	[141]
		NAC	Antioxidant	[142]
Epigenetic reprogramming	Histone methylation	DZNep	Rescuing endogenous MSC defects and promoting bone	[144]
	regulators	Pargyline	regeneration in osteoporosis and fracture healing	[145]
		JIB-04		[146]
		microRNAs	Regulating the endogenous expressions of multiple growth factors simultaneously	[68,147–150,259–261]
		EVs	Restoring bone homeostasis	[53,68,259]
Pre-conditioning on MSCs	Pharmaceutics:	Icariin	Loaded on calcium phosphate cement scaffolds to promote the osteogenesis potential of endogenous MSCs	[135]
		Heparin	Reducing thrombosis and improving heart infarct after acute intracoronary MSCs delivery	[205]
		Melatonin	Improving MSCs osteogenesis as stemness preserver	[54]
		Aspirin	Enhancing resistance of MSCs in recipient inflammatory microenvironment	[52,217]
Microenvironment-based strategies				
Alternative cells	MSCs	ADMSCs	Replenishing resident MSCs and modifying the recipient systemic	[181]
		DPSCs	microenvironments to further enhance endogenous regeneration	[185]
		PDLSCs		[189]
		GMSCs		[194]
		UCMSCs		[243]
Pre-conditioning	Pharmaceutics	Aspirin	Inhibiting regional IFN-γ and TNF-α levels	[52]
		Melatonin	ROS eliminator and intracellular signaling and immune regulator.	[210–215]

SDF-1——Stromal cell-derived factor-1; TGFβ3——Transforming growth factor β3; CTGF——Connective tissue growth factor; NF-κB—— Nuclear transcription factor-kappa B; DPSCs, ——Dental pulp stem cells; GMSCs——Gingival Mesenchymal Stem Cells; PDLSCs——Periodontal ligament stem cells; ROS—— Reactive oxygen species; EVs——extracellular vesicles.

abolish osteogenic impairments of BMMSCs and could rescue resident BMMSCs deficiency in estrogen-deficient condition [139,151].

4.1.2. MSCs transplantation

It has been documented that both systemically and locally transplanted MSCs induce recipient cellular responses (other than immune cells) in bone regeneration [153,154]. Particularly, upon homing to bone marrow, infused MSCs could promote recipient osteoblastogenesis in preventing glucocorticoid-induced bone loss [153]. Other than harnessing endogenous MSCs in bone healing, transplantation of exogenous MSCs is becoming a new paradigm of MSC cell therapy with the initial aim to replenish resident MSCs and take better advantage of their bone regenerative potential, as this approach showed immense immunomodulatory potential to modify the recipient systemic microenvironments and to further enhance endogenous regeneration [155].

One of the most profound interplay between transplanted MSCs and microenvironment is the re-establishment of recipient immunological balance, particularly through restoring the homeostasis among T-cell subsets by suppressing the pro-inflammatory T helper 1 (Th1) and Th17 cells while promoting the anti-inflammatory Th2 and the $CD4^+CD25^+Foxp3^+$ T_{regs} [156,157] and maintaining macrophage homeostasis by promoting the anti-inflammatory M2 polarization and inhibiting the pro-inflammatory M1 polarization [158]. Within the interests of regenerative medicine, MSCs from several tissue materials have mainly been utilized in the field of bone regeneration including bone marrow, adipose tissue, umbilical cord, and, recently, clinically discarded dental-related tissues that have long been considered to be of no use as shown in Table 5 [159–166].

3.1.2.1. Bone marrow mesenchymal stem cells. Intravascular injection of BMMSCs has proved to be a promising treatment for autoimmune diseases [167,168], vascular diseases [169], graft vs. host disease (GVHD) [170] and diabetes [171]. Indeed, bone marrow has been directly applied to induce bone formation in skeletal defects and non-unions. BMMSCs have been used in bone regeneration research for at least four decades [172] and their validity has been examined from various perspectives. Now, BMMSC cell therapy clinical trials are being carried out on systemic and local bone regeneration world-wide (https://www.clinicaltrials.gov/.), but their function are prone to pathological factors of bone, limiting the application of autologous cells [72]. Moreover, shortage of bone marrow donation, invasive isolation procedure for donors and patients, extreme low ratio of MSCs in tissue cells and poor multipotent ability after extensive passage or at aged people constrain the feasibility of using them in large commercial applications [30,173].

3.1.2.2. Adipose-derived mesenchymal stem cells. Adipose tissue has recently been attracting attention as an alternative source of MSCs and particularly are believed to be applicable cell sources as they can be simply harvested in large scale with less donor site morbidity [174].

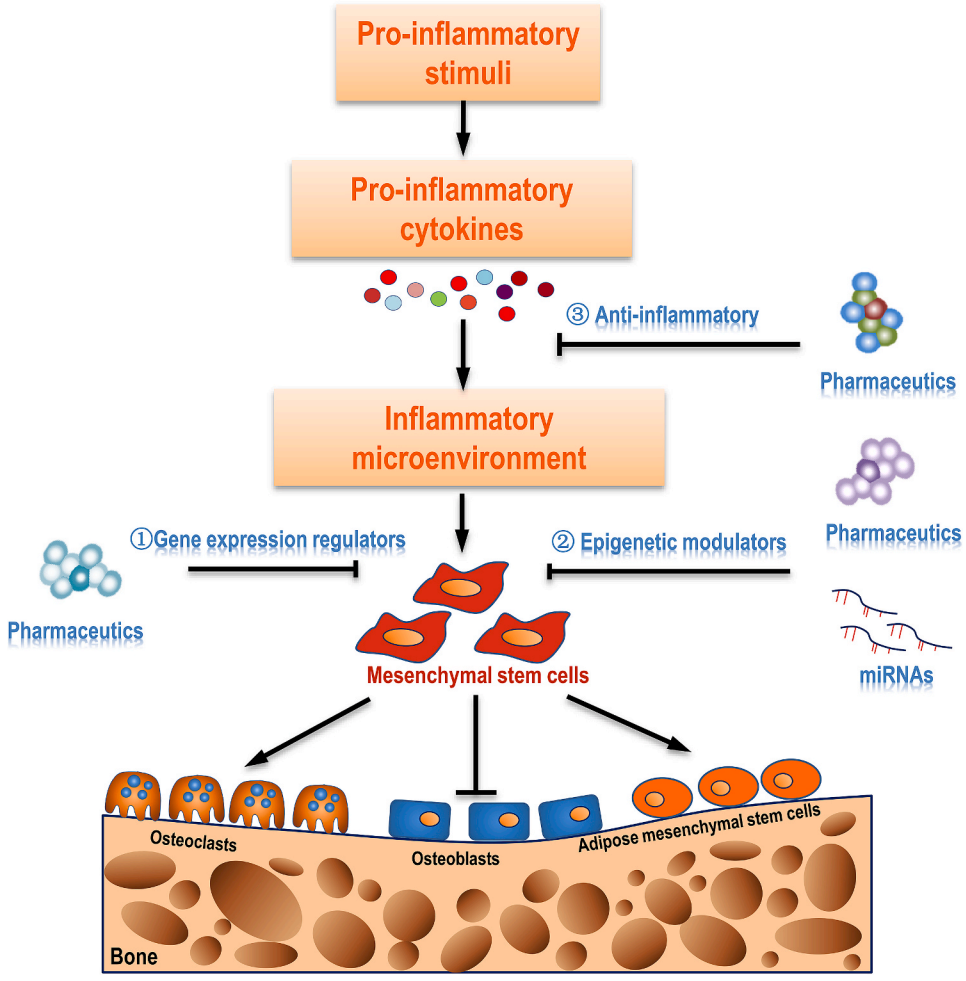

Fig. 4. Inflammatory microenvironment impairs resident MSCs and solutions.

ADMSCs firstly reported by Zuk et al. [175] demonstrated to have self-renewal ability and multilineage differentiation potential [176]. Moreover, the cell yield of ADMSCs from adipose tissue is higher than BMMSCs from bone marrow aspirates [177]. These cells also have a several benefits in vitro: faster and easier expansion in culture, more passage cells that retain stem cell phenotypes, pluripotency and less susceptibility to age [178,179]. Moreover, they have a low risk of rejection [180] and preserved therapeutic efficacy in locoregional bone regeneration [181]. Although numerous studies have reported that ADMSCs may present similar or even higher regenerative ability compared to BMMSCs, "Is the osteogenic capacity of ADMSCs the same or far better than BMMSCs?" is still controversial now followed by comparing the osteogenic capacity in vitro and bone regeneration ability in vivo between them based on the literature which utilized both BMMSCs and ADMSCs simultaneously in their articles [30].

3.1.2.3. Dental related MSCs. Dental tissues are obtained during standard dental procedures and discarded as surgical waste products; hence harvesting cells from these tissues do not raise ethical concerns but make them attractive for therapeutic purposes. MSCs can be isolated with ease from various dental tissues including dental pulp stem cells, periodontal ligament stem cells and gingival stem cells and so on [182]. Dental tissues have gained attention since their first isolation from dental pulp in 2000 [183] due to their high accessibility and multilineage differentiation capacity [184].

Compared to BMMSCs, dental pulp stem cells (DPSCs) exhibited higher CFU-F and proliferation rates along with a similar gene expression profile for genes related to mineralization [185,186]. However, DPSCs transplants formed dentin-pulp like structure by ex vivo expansion whereas ectopic bone formation was present for BMMSCs transplants in vivo [187]. Immunomodulatory properties of periodontal ligament stem cells (PDLSCs) also revealed PDLSCs could as candidates for allogeneic stem cell-based therapies [188]. Subsequent investigations demonstrated that PDLSCs [28] showed slow differentiation into osteoblasts in vivo, compared to BMMSCs in the treatment of calvarial bone defects [189]. Considered as a novel postnatal stem cell source, availability of gingival mesenchymal stem cells (GMSCs) is abundance as they are easily obtained from donor gingival tissue [190,191]. GMSCs display stable morphology along with high proliferation rates compared to BMMSCs, sustain MSC characteristics and maintain karyotype and telomerase activity during prolonged culture time and immunomodulatory effects [191–193]. GMSCs were reported to successfully regenerate boney defects in the Mandible and Calvaria of rats by their direct participation in bone formation, and the recruitment of bone progenitor cells [194]. A single systemic infusion of GMSCs was also successful in causing a significant improvement in the osteoporotic bone phenotype caused by estrogen deficiency and immune-mediated effect of GMSCs in the OVX mice is through PD-L1 immune checkpoint and immune tolerance led by the influence of transplantation on T-cell populations [195]. Despite tremendous amount of preclinical studies, very few clinical trials evaluated dental stem cells for bone tissue engineering except for DPSCs and PDLSCs. But, limited cell availability of dental stem cells and donor age are two main challenges faced in clinical applications as it affects regenerative properties of stem cells.

Table 5
The characteristics of several types of mesenchymal stem cells in bone regeneration.

Source	Mesenchymal stem cells	Found	Advantages	Disadvantages
Bone marrow	BMMSCs	Friedenstein et al., in 1987 [63]	Directly inducing bone formation and have been used in bone regeneration research for at least four decades [172]	Shortage donation, invasive isolation procedure, extreme low cell yield ratio and poor multipotent ability after extensive passage or at aged people [30,173]
Adipose tissue	ADMSCs	Zuk et al., in 2001 [175]	Simply harvested procedure in large scale with less donor site morbidity, higher cell yield ratio, faster and easier expansion in culture, retained stem cell phenotypes, pluripotency at extensive passage or aged and a low risk of rejection [174,177–180].	Whether the osteogenic capacity of ADMSCs is same or far better than BMMSCs remains controversial [30]
Dental related tissue	DPSCs	Shi et al., in 2000 [185]	Without ethical concerns, higher accessibility, higher proliferation rates, along with a similar gene expression profile for genes related to mineralization [185,186].	Forming dentin-pulp like structure compared to BMMSCs [187]
	PDLSCs	Shi et al., in 2004 [28]	Without ethical concerns, but with immunomodulatory properties [188]	Slow differentiation into osteoblasts in vivo compared to BMMSCs [189]
	GMSCs	Shi et al., in 2009 [191]	Abundant availability, sustained stem cell characteristics during prolonged culture time, higher proliferation rates and immunomodulatory effects [191–193].	Regenerative properties limited by donor age in clinical applications
Umbilical cord tissue	UCMSCs	Wang et al., in 2004 [242]	Without ethical concerns, higher accessibility, plasticity and developmental flexibility, minimal immunorejection and tumorigenicity [162,163,242].	Lower differentiation into osteoblasts.

BMMSCs——bone marrow mesenchymal stem cells; ADMSCs, adipose-derived mesenchymal stem cells.
DPSCs——dental pulp stem cells; PDLSCs——periodontal ligament stem cells; GMSCs——gingival Mesenchymal Stem Cells; UCMSCs——umbilical cord mesenchymal stem cells.

4.1.3. Premodified MSCs infusion

Safety and efficacy are two essential factors to be considered for clinical treatment. Normally, 1–2 × 10^6/kg BMMSCs were administered intravenously into patients once within a short time in most clinical cases [196,197]. Such large quantity of BMMSCs leads to a number of issues, such as adverse effects induced by the infused BMMSCs and in vitro cell expansion [128]. Moreover, a number of acute adverse events, such as fever [128], instant blood-mediated inflammatory reaction [198], microvascular embolism [198–200] and impaired heart function [201–203] have been induced after BMMSCs injection. Notably, two cases of sudden death caused by lung embolism after MSCs infusion focused worldwide attention on the acute toxicity of MSCs [204,205]. Therefore, identifying strategies to improve the therapeutic safety and effect is becoming a crucial issue for BMMSC cell therapy. Indeed, safety and therapeutic effect for both local and systemic infusion of BMMSCs therapy could be improved in the clinic with the effect of anticoagulation treatment though heparin, a widely used safe drug, which could reduce thrombosis and improve heart infarct after acute intracoronary MSCs delivery in acute myocardial infarction [205,206]. Meanwhile, various problems are also encountered during in vitro expansion as described in 4.1.2. Notably, a number of disorders of MSCs have been reported to be accompanied with long-term in vitro passaging [207,208]. Not only MSCs derived from diseased donors, but allogeneic MSCs (from healthy individuals) could also exhibit inhibited capability in bone regeneration after long-term culture and passages in vitro [54]. In consideration to utilize pharmacologic action to optimizing MSCs application, melatonin, a metabolic regulator [48], has been proved that could improve MSCs osteogenesis by preserving stemness [54,209]. It also promoted MSCs-based local bone regeneration in both ectopic sites and critical-sized calvarial bone defects [54] as a compound with great potential of reactive oxygen species (ROS) elimination [210], intracellular signaling regulation [211], senescence delaying, ossification promotion and immune regulation [212–215].

The recipient microenvironmental statuses also greatly influence the therapeutic performance of MSCs, thus it has gradually been noticed that diseased microenvironment constitutes major barriers limiting bone regeneration. Pathologically, a variety of inflammatory cytokines is capable of inducing extensive bone loss, thus, it is now generally appreciated that crosstalk among inflammatory cells and cells related to bone healing is essential to the formation repair and remodeling of bone [216]. The control of transplanted MSCs efficacy by recipient inflammatory microenvironment also exists in systemic MSC cytotherapy, despite more complicating. Accordingly, pre-conditioning on MSCs has been reported effective in resisting recipient diseased microenvironmental impacts and improving regenerative potential. Aspirin could be used to pretreat allogeneic MSCs before them being transplanted in vivo, demonstrating enhanced resistance in recipient inflammatory microenvironment. This aspirin preconditioning significantly promoted MSCs-based ectopic bone regeneration [52,217]. In local MSCs transplantation, aspirin can also be applied in a site-specific manner around the MSCs transplanted location through inhibiting regional IFN-γ and TNF-α levels in MSC implants to promote MSC mediated healing of critical-sized calvarial bone defects [52].

4.2. MSCs based biomaterials technique

Therapies with the potential to become routine in the clinic will only be possible if these methods ensure efficient engraftment and the survival of a therapeutically relevant number of cells [218]. The bone tissue engineering approach is still in continuous progresses by promoting MSCs viability with MSCs based biomaterials technique. In addition to considerations of stem cells that may favor bone repair, the delivery strategy also plays an essential part in the design of cell-based tissue regeneration. A conventional technique involving single cell suspension injection showed compromised promoting effects on tissue regeneration, but with low cell activity, uneven distribution and low

regeneration efficiency [219]. So, developing a novel bone transplanting system is necessary for basic research and clinical work in the field of bone regeneration. Indeed, endogenous MSCs reside in a complex architecture composed of neighboring cells and abundant neurovascular bundles, in which MSCs behaviors are tightly controlled by the local niche according to requirements of the host tissues [220–222]. Scientists have learned this fact and that the use of human-derived biomaterial scaffolds, naturally occurring proteins, ECM components and preparations rich in growth factors or non-expanded stem cells for tissue engineering provide new approaches for the redesign of clinically translatable regenerative therapies, which has been stated in 3.2. Moreover, in the process of cell harvesting, proteolytic enzymes would deteriorate the ECM and growth factors of seed cells which play an important role in forming bone formation microenvironment [223,224]. For optimizing application of exogenous MSCs in bone tissue engineering, technique of cell-sheets or -aggregates engineering would mostly reserve the ECM which could be considered as a scaffold tissue so that the cell-sheets or -aggregates themselves would be used as transplantable tissue [32,33,225]. Compared to cell suspension technology, cell-sheets or -aggregates could significantly improve cell retention, vascular density and graft-host cell connection in the transplanting area [226].

Cell-sheets technology could preserve the self-produced tissue-specific extracellular matrix thus mimicking natural microenvironments in terms of various mechanical, chemical, and biological properties and the immense regenerative potential of which is proved in various tissues including skin and periodontium [227,228]. Concerted efforts have been and still are being made to harvest the living cell sheets more easily and effectively to avoid a relatively complicated, time consuming sheets grafting procedure [229,230]. Specifically, Wei et al. (2012) developed a new, simple, and practical approach to generate vitamin C (Vc)-induced PDLSCs sheets, leading to up regulated expression of ECM proteins and typical osteogenic markers in the resultant cell sheets [231]. Then, Shang et al. polished the preparation techniques for cell-sheets, which named as cell-aggregates [24]. In cell aggregates biotechnology, the ECM produced by cells includes numerous signals that influence cellular activities, and coordinated interactions between soluble factors, grafting cells, and extracellular matrices define a local biochemical and mechanical niche with complex and dynamic regulation that determines the structure and function of a newly formed tissue. Therefore, enhancing the ability of targeted cells to preserve and generate ECM is helpful for tissue regeneration [232]. In addition, cell-aggregates technology makes it easier to detach the cells from the culture substrate, so that the natural adhesion molecules on the cell surface and cell-cell interactions remain intact [232–234]. This technology could establish topical favorable microenvironments and has been recognized as a promising concept for cell delivery with adhesion molecules on the cell surface. Indeed, as a self-assembly approach to recreate the regenerative microenvironment with specific natural condition, MSCs-aggregates have been successfully established and used to repair large defects of bone [25,231].

Despite that bone tissue engineering has achieved great advance in healing of regional bone defects, bone regeneration in osteoporotic bone defects and fractures remain as an unfulfilled challenge. In this regard, CBB-BMMSCs-sheets composite material in repairing large area cranial bone defects in osteoporosis rats with BMMSCs isolated from allograft normal rats demonstrating that the CBB-BMMSCs-sheets composite materials had a more pronounced potential of bone regeneration and reconstruction in healing bone defects than CBB-BMMSCs composite material did in osteoporosis rats in vivo and in vitro. CBB-BMMSCs-sheets composite materials also could repair large area bone defects by newly formed bone tissues with force bearing characteristic in a short time both in normal or osteoporosis state [25]. Moreover, a wide range of chemical compounds derived from Chinese herbs that have been used as tonics and aphrodisiacs and for the treatment of bone-related diseases have been investigated as therapeutic agents and possible mediators of the regulatory processes of adult stem cell differentiation and their movement in tissue repair and rejuvenation [235–239]. Active ingredients and compounds derived from Chinese herbs are able to efficiently regulate stem cell fate, proliferation, and differentiation, thus, the use of which has opened a new avenue for the stimulation and acceleration of wound healing and tissue regeneration. In this regard, licochalcone A, a small molecular compound derived from licorice root, has been identified to further promote bone regenerative potential of MSCs-aggregates, and the application of which facilitated extracellular matrix secretion and osteogenic differentiation of MSCs-aggregates. This biomodified cell-aggregates enhanced bone formation in treating metaphyseal defects of estrogen-deficient recipients [24]. Moreover, Osthole has been proved that it can enhance bone-forming activity directly by triggering cell osteogenic differentiation and bone marker gene expression [240], induce new bone formation and prevent bone loss caused by estrogen deficiency [241]. The anabolic effect of stimulating osteoblast differentiation of cell sheets has also been confirmed in the ectopic in vivo transplantation model [240]. Other than pharmacological based modified donor MSCs, resistance to diseased microenvironment might also be achieved by using different sources of MSCs. Among these, human umbilical cord mesenchymal stem cells (hUCMSCs) derived from umbilical cords, are an inexpensive and inexhaustible stem cell source [242]. Their harvest does not require the invasive procedure and does not have the controversies of human embryonic stem cells [162]. Furthermore, hUCMSCs appeared to be primitive MSCs and exhibit high plasticity and developmental flexibility [163]. In addition, hUCMSCs have demonstrated minimal immunorejection in vivo and are not tumorigenic [163]. It has been reported that hUCMSCs derived cell-aggregates repaired periodontal tissue defects including hard tissues under inflammatory periodontitis condition [243]. The above approaches constitute current MSCs-based cell-sheets or -aggregates strategies to optimize bone healing in diseased microenvironments.

4.3. Cell-free strategies

Living cell transplantation may cause more safety concerns as it closely related to tumor and emboli formation [244]. Other than participating in tissue regeneration by themselves directly, MSCs possess powerful release capability to secrete various cytokines and extracellular vesicles (EVs) as masters of cell-cell shuttle of biomolecules such as RNA, miRNAs and protein “cargos” to modulate the ambient microenvironmental property [53,155,245,246]. Notably, exosomes (also called nanovesicles; size within 100 nm) are the best studied EV subpopulation and have been elaborated that mediate MSCs-based bone regeneration in healing of bone defects [247–249]. Ascending evidence indicates that exosomes represent the most prominent form of EV signaling in mediating MSCs-mediated bone healing even in recapitulating MSCs therapeutic effects on osteoporosis, where systemic application of MSCs-derived exosomes restore bone mass as efficiently and long-lasting as MSCs themselves. Mechanistically, donor exosomes secreted by transplanted MSCs ameliorate resident BMMSCs deficiency via transfer of miRNAs and proteins, regulating recipient epigenetic states to mediate the sustainable effects [53,68]. From a translational perspective, EVs could easily be sustainably and reproducibly obtained and stored, and may offer specific advantages of privilege and safety to recipient microenvironments. As stated above, EVs-based cell-free therapies has considerable translational potential in developing “cell-free” therapy for regenerative therapy as novel alternatives or even superior to the whole-cell MSCs therapy [250,251]. Particularly, recent preclinical studies suggest that the MSCs secretome (the EVs/exosomes released by MSCs) has been successfully applied in enhancing calvarial, femoral and periodontal bone regeneration [247,252,253].

Pathologically, EVs with high stability lipid bilayer in vivo [250] could facilitate bone fractures healing and osteoporosis induced by diabetes and estrogen deficiency [254,255] and demonstrate long-term

therapeutic effects on recipient MSCs deficiency via epigenetic reprogramming thus persistently restoring bone homeostasis in recipient diseased microenvironments [53,68]. Exosomes also have shown bone therapeutic potential in diseased donor microenvironments in treating osteonecrosis and in repairing critical-sized bone defects in estrogen-deficient mice [249,253]. The exosome resistance to recipient diseased microenvironments has further been confirmed in the maintenance of therapeutic effects in treating osteoporosis in autoimmune conditions of systemic lupus erythematosus (SLE) and systemic sclerosis (SS) [53,68]. Based on current information available, it can be assumed that translation of EV- based cell-free therapies as a feasible clinical approaches in bone healing will thrive that promises a significant amount of preclinical and clinical investigations in the near future. However, there are still some core challenges have not yet been overcome that restricted the development of exosomes into therapeutically agents for clinical usage, including low yielding [256], unreliable isolation method [257] and unprofiling and inadequate understanding of exosomal contents and the underlying mechanisms [258].

Compared to EV- based cell-free therapies, epigenetic reprogramming has also been performed long-lasting resistance to recipient microenvironmental impacts on MSCs via application of the post-transcriptional molecules. miRNAs are a large class of small, non-coding RNAs that function as repressors of gene expression at the level of posttranscriptional regulation and have been found to play important roles in most known animal genomes. As a single miRNA is often involved in several gene regulatory networks, overexpression or inhibition of miRNA can regulate the endogenous expressions of multiple growth factors simultaneously. Here, our lab constructed chimeric apoptotic bodies (cABs) for on-demand inflammation modulation by combining pure membrane from apoptotic bodies (ABs) as a bioconjugation/regulation module and mesoporous silica nanoparticles (MSNs) as a engineered EVs carrying therapeutic molecules, which are regarded as promising candidates for disease therapies. MSNs were preloaded with anti-inflammatory agents (microRNA-21 or curcumin) and modified with stimuli-responsive molecules to achieve accurate cargo release at designated locations. The resulting cABs actively target macrophages in the inflammatory region and effectively promote M2 polarization of these macrophages to modulate inflammation due to the synergistic regulatory effects of AB membranes and the intracellular release of preloaded cargos. Both the in vitro and in vivo results showed that our engineered EVs are able to modulate cutaneous inflammation, promote regeneration, and ameliorate the inflammatory bowel diseases, demonstrating that this method may be an efficient strategy to engineer EVs in a modularized way for various biomedical applications [259]. Moreover, given the potent function of miR-26a in promoting angiogenic-osteogenic coupling of MSCs, it was therefore hypothesized that delivery of a desired miRNA may result in optimization of bone regeneration through coordinating of endogenous angiogenesis and osteogenesis process. Initially, a miRNA enhancer delivery system releasing agomiR-26a were applied with MSCs, in a localized and sustained manner in vivo, leading to complete repair of critical-sized calvarial bone defects with increased vascularization [260]. Furthermore, miR-26a overexpression has been proved that could restore the impaired function of estrogen-deficient induced MSCs and repair critical-sized calvarial bone defects [261].

5. Conclusions

Although it remains a great challenge that how to achieve bone loss and defects therapeutic efficacy, MSCs-based bone regeneration including cytotherapy, cell sheets/aggregates technique, and cell free approaches still holds great promise for curing severe large bone defects no matter in regular or in diseased microenvironments. In fact, the critical function of transplanted MSCs relies not only on the replenishment of the number of resident MSCs to recovery the potential of bone healing, but also on the profound impact of MSCs derived molecular components mediating and modulating efficacy of themselves and microenvironment even in skeletal conditions. Moreover, the survival of systemically infused MSCs in recipients is not endurable or affected by diseased microenvironmental factors which have been indicated recently using labeling by either green fluorescent protein (GFP) or fluorescent dyes [51]. While, there are still other many limitations to be solved in the pre-clinical or clinic translation of MSCs-mediated bone healing, especially in diseased microenvironments.

In abnormal conditions, some novel bioactive molecules such as anticoagulation treatment to reduce thrombosis, certain pharmacies with the role of preserving stemness of transplanted MSCs and anti-inflammatory to pre-normalizing recipient microenvironment have shown the promising opportunities and solutions for cell viability and regulation of cell osteogenic differentiation to optimize MSCs-mediated bone healing. Increasing evidence also suggests that the idea of creating beneficial microenvironments for MSCs-based bone regeneration has shown promise for future practice of bone healing. With the appearance and improvement of cell-sheets/-aggregates techniques, more sophisticated cell-sheets/-aggregates for bone regeneration have been established by biomodified through pharmaceutics processed and seed cells substitutes to fulfill osteoporotic bone healing.

Notably, EVs derived from MSCs served as “cargos” to modulate the microenvironmental and miRNAs acted as posttranscriptional molecules to achieve epigenetic reprogramming, have attracting increasing attention as novel cutting-edge strategies in bone healing, in which the cell-free therapeutic options based on MSCs-derived EVs further pushing forward bone regeneration and will shed light on the exciting option to bone healing in aging and diseases [262]. Although there is currently limited evidence to confirm MSCs based bone regeneration capacity in diseased condition, especially MSCs based cell-sheets/-aggregates techniques and cell free strategies, these promising field can attract further investigations which are required to confirm the clinical effects of these approaches enumerated ahead in the regenerative treatment of bone tissues.

Declaration of competing interest

The authors declare no competing financial interests.

Acknowledgements

This research was supported by the National Key Research and Development Program of China (2016YFC1101400), the National Natural Science Foundation of China (No. 81930025, 81620108007, 81901010), and Open Foundation of Shandong Provincial Key Laboratory of Oral Tissue Regeneration (No. SDKQ201904).

Abbreviations

3D	three-dimensional
ABs	apoptotic bodies
ADMSCs	adipose-derived mesenchymal stem cells
ADM	adipose-derived mesenchymal stem cells derived ECM
bdECM	bone decellularized ECM
BMMSCs	bone marrow mesenchymal stem cells
BMP-2	bone morphogenetic protein-2
BMP-7	bone morphogenetic protein-7
BMPs	bone morphogenetic proteins
cABs	chimeric apoptotic bodies
CBB	calcined bovine bone
CTGF	Connective tissue growth factor
DBM	demineralized bone matrix
DPSCs	dental pulp stem cells
ECM	extracellular matrix
ESCs	Embryonic Stem Cells

EVs	extracellular vesicles
FBS	fetal bovine serum
GFP	green fluorescent protein
GMSCs	gingival Mesenchymal Stem Cells
GVHD	graft vs. host disease
HCl	hydrogen chloride
HSCs	hematopoietic stem cells
hUCMSCs	human umbilical cord mesenchymal stem cells
IFN-γ	interferon-gamma
IL-10	interleukin-10
miRNAs	microRNAs
MSCs	Mesenchymal stem cells
MSNs	mesoporous silica nanoparticles
NCPs	non-collagen proteins
NF-κB	factor-kappaB
NF-gelatin	nanofibrous gelatin
OVX	ovariectomized
PDLSCs	periodontal ligament stem cells
PL	platelet lysate
ROS	reactive oxygen species
SDF-1	stromal cell-derived factor-1
SLE	systemic lupus erythematosus
SS	systemic sclerosis
TGF β3	transforming growth factor β3
Th1	T helper 1
TNF-α	tumor necrosis factor-alpha
Tregs	regulatory T cells
Vc	vitamin C

Author contributions

F.Q.S, Y.Y and S.Y.L contributed equally to the study design, manuscript preparation and revision. L.G.M., Y. J,Z, Z.F.Z., and J.Y.Z made important intellectual contribution to the manuscript revision. Y.J. conceived and supervised the study. All authors have reviewed and approved the final version of the manuscript.

References

[1] U. Thormann, S. Ray, U. Sommer, T. Elkhassawna, T. Rehling, M. Hundgeburth, A. Henß, M. Rohnke, J. Janek, K.S. Lips, C. Heiss, G. Schlewitz, G. Szalay, M. Schumacher, M. Gelinsky, R. Schnettler, V. Alt, Bone formation induced by strontium modified calcium phosphate cement in critical-size metaphyseal fracture defects in ovariectomized rats, Biomaterials 34 (2013) 8589–8598, https://doi.org/10.1016/j.biomaterials.2013.07.036.

[2] G.C. Gurtner, M.J. Callaghan, M.T. Longaker, Progress and potential for regenerative medicine, Annu. Rev. Med. 58 (2007) 299–312, https://doi.org/10.1146/annurev.med.58.082405.095329.

[3] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound repair and regeneration, Nature 453 (2008) 314–321, https://doi.org/10.1038/nature07039.

[4] Z. Guo, S. Iku, L. Mu, Y. Wang, T. Shima, Y. Seki, Q. Li, Y. Kuboki, Implantation with new three-dimensional porous titanium web for treatment of parietal bone defect in rabbit, Artif. Organs 37 (2013) 623–628, https://doi.org/10.1111/aor.12058.

[5] S.C. Gamradt, J.R. Lieberman, Bone graft for revision hip arthroplasty: biology and future applications, Clin. Orthop. Relat. Res. (2003) 183–194, https://doi.org/10.1097/01.blo.0000096814.78689.77.

[6] R. Zimmermann, R. Jakubietz, M. Jakubietz, E. Strasser, A. Schlegel, J. Wiltfang, R. Eckstein, Different preparation methods to obtain platelet components as a source of growth factors for local application, Transfusion 41 (2001) 1217–1224, https://doi.org/10.1046/j.1537-2995.2001.41101217.x.

[7] M.A. Schallenberger, K. Rossmeier, H.M. Lovick, T.R. Meyer, H.M. Aberman, G.A. Juda, Comparison of the osteogenic potential of OsteoSelect demineralized bone matrix putty to NovaBone calcium-phosphosilicate synthetic putty in a cranial defect model, J. Craniofac. Surg. 25 (2014) 657–661, https://doi.org/10.1097/SCS.0000000000000610.

[8] T. Yoshikawa, H. Ohgushi, S. Tamai, Immediate bone forming capability of prefabricated osteogenic hydroxyapatite, J. Biomed. Mater. Res. 32 (1996) 481–492, https://doi.org/10.1002/(SICI)1097-4636(199611)32:3<481::AID-JBM23>3.0.CO;2-I.

[9] S.S. Jensen, N. Broggini, G. Weibrich, E. Hjôrting-Hansen, R. Schenk, D. Buser, Bone regeneration in standardized bone defects with autografts or bone substitutes in combination with platelet concentrate: a histologic and histomorphometric study in the mandibles of minipigs, Int. J. Oral Maxillofac. Implants 20 (2005) 703–712.

[10] M. Piattelli, G.A. Favero, A. Scarano, G. Orsini, A. Piattelli, Bone reactions to anorganic bovine bone (Bio-Oss) used in sinus augmentation procedures: a histologic long-term report of 20 cases in humans, Int. J. Oral Maxillofac. Implants 14 (1999) 835–840.

[11] J.W. Vehof, P.H. Spauwen, J.A. Jansen, Bone formation in calcium-phosphate-coated titanium mesh, Biomaterials 21 (2000), https://doi.org/10.1016/s0142-9612(00)00094-6 2003–2009.

[12] J.A. Burdick, L.M. Philpott, K.S. Anseth, Synthesis and characterization of tetrafunctional lactic acid oligomers: a potential in situ forming degradable orthopaedic biomaterial, J. Polym. Sci. Polym. Chem. 39 (2001) 683–692.

[13] J. Shi, J. Sun, W. Zhang, H. Liang, Q. Shi, X. Li, Y. Chen, Y. Zhuang, J. Dai, Demineralized bone matrix scaffolds modified by CBD-SDF-1α promote bone regeneration via recruiting endogenous stem cells, ACS Appl. Mater. Interfaces 8 (2016) 27511–27522, https://doi.org/10.1021/acsami.6b08685.

[14] R.E. Marx, E.R. Carlson, R.M. Eichstaedt, S.R. Schimmele, J.E. Strauss, K.R. Georgeff, Platelet-rich plasma: growth factor enhancement for bone grafts, Oral, Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod. 85 (1998) 638–646, https://doi.org/10.1016/s1079-2104(98)90029-4.

[15] H. Schilephake, Bone growth factors in maxillofacial skeletal reconstruction, Int. J. Oral Maxillofac. Surg. 31 (2002) 469–484, https://doi.org/10.1054/ijom.2002.0244.

[16] C.H. Heldin, B. Westermark, Mechanism of action and in vivo role of platelet-derived growth factor, Physiol. Rev. 79 (1999) 1283–1316, https://doi.org/10.1152/physrev.1999.79.4.1283.

[17] K. Sugimoto, S.P. Gordon, E.M. Meyerowitz, Regeneration in plants and animals: dedifferentiation, transdifferentiation, or just differentiation? Trends Cell Biol. 21 (2001) 212–218, https://doi.org/10.1016/j.tcb.2010.12.004.

[18] B.C. Kim, H. Bae, I.K. Kwon, E.J. Lee, J.H. Park, A. Khademhosseini, Y.S. Hwang, Osteoblastic/cementoblastic and neural differentiation of dental stem cells and their applications to tissue engineering and regenerative medicine, Tissue, Eng. Part. B. Rev. 18 (2012) 235–244, https://doi.org/10.1089/ten.TEB.2011.0642.

[19] M. La Noce, F. Paino, A. Spina, P. Naddeo, R. Montella, V. Desiderio, A. De Rosa, G. Papaccio, V. Tirino, L. Laino, Dental pulp stem cells: state of the art and suggestions for a true translation of research into therapy, J. Dent. 42 (2014) 761–768, https://doi.org/10.1016/j.jdent.2014.02.018.

[20] S. Wang, X. Qu, R.C. Zhao, Clinical applications of mesenchymal stem cells, J. Hematol. Oncol. 5 (2012) 19, https://doi.org/10.1186/1756-8722-5-19.

[21] P.D. Potdar, Y.D. Jethmalani, Human dental pulp stem cells: applications in future regenerative medicine, World, J. Stem Cell. 7 (2015) 839–851, https://doi.org/10.4252/wjsc.v7.i5.839.

[22] M. Aljamie, L. Alessa, R. Noah, L. Elsayed, Dental pulp stem cells, a new era in regenerative medicine: a literature review, Open J. Stomatol. 6 (2016) 155–163, https://doi.org/10.4236/ojst.2016.66020.

[23] P. Potdar, J. Sutar, Establishment and molecular characterization of mesenchymal stem cell lines derived from human visceral & subcutaneous adipose tissues, J. Stem Cells Regen. Med. 6 (2010) 26–35.

[24] F. Shang, L. Ming, Z. Zhou, Y. Yu, J. Sun, Y. Ding, Y. Jin, The effect of licochalcone A on cell-aggregates ECM secretion and osteogenic differentiation during bone formation in metaphyseal defects in ovariectomized rats, Biomaterials 35 (2014) 2789–2797, https://doi.org/10.1016/j.biomaterials.2013.12.061.

[25] Y. Liu, L. Ming, H. Luo, W. Liu, Y. Zhang, H. Liu, Y. Jin, Integration of a calcined bovine bone and BMSC-sheet 3D scaffold and the promotion of bone regeneration in large defects, Biomaterials 34 (2013) 9998–10006, https://doi.org/10.1016/j.biomaterials.2013.09.040.

[26] P.D. Potdar, S.B. D'souza, Isolation of Oct4+, Nanog+ and SOX2- mesenchymal cells from peripheral blood of a diabetes mellitus patient, Hum. Cell 24 (2011) 51–55, https://doi.org/10.1007/s13577-011-0011-6.

[27] P. Potdar, R. Subedi, Defining molecular phenotypes of mesenchymal and hematopoietic stem cells derived from peripheral blood of acute lymphocytic leukemia patients for regenerative stem cell therapy, J. Stem Cells Regen. Med. 7 (2011) 29–40.

[28] B.M. Seo, M. Miura, S. Gronthos, P.M. Bartold, S. Batouli, J. Brahim, M. Young, P.G. Robey, C.Y. Wang, S. Shi, Investigation of multipotent postnatal stem cells from human periodontal ligament, Lancet 364 (2004) 149–155, https://doi.org/10.1016/S0140-6736(04)16627-0.

[29] P.D. Potdar, Y.D. Jethmalani, Human dental pulp stem cells: applications in future regenerative medicine, World, J. Stem Cell. 7 (2015) 839–851, https://doi.org/10.4252/wjsc.v7.i5.839.

[30] H.T. Liao, C.T. Chen, Osteogenic potential: comparison between bone marrow and adipose-derived mesenchymal stem cells, World, J. Stem Cell. 6 (2014) 288–295, https://doi.org/10.4252/wjsc.v6.i3.288.

[31] A.N. Renth, M.S. Detamore, Leveraging "raw materials" as building blocks and bioactive signals in regenerative medicine, Tissue Eng. B Rev. 18 (2012) 341–362, https://doi.org/10.1089/ten.TEB.2012.0080.

[32] K. Bittner, P. Vischer, P. Bartholmes, P. Bruckner, Role of the subchondral vascular system in endochondral ossification: endothelial cells specifically derepress late differentiation in resting chondrocytes in vitro, Exp. Cell Res. 238 (1998) 491–497, https://doi.org/10.1006/excr.1997.3849.

[33] J. Yang, M. Yamato, C. Kohno, A. Nishimoto, H. Sekine, F. Fukai, T. Okano, Cell sheet engineering: recreating tissues without biodegradable scaffolds, Biomaterials 26 (2005) 6415–6422, https://doi.org/10.1016/j.biomaterials.2005.04.061.

[34] F.M. Watt, B.L. Hogan, Out of Eden: stem cells and their niches, Science 287 (2000) 1427–1430, https://doi.org/10.1126/science.287.5457.1427.

[35] A.R. Vaccaro, H.A. Stubbs, J.E. Block, Demineralized bone matrix composite grafting for posterolateral spinal fusion, Orthopedics 30 (2007) 567–570, https://

doi.org/10.3928/01477447-20070701-06.
[36] H.N. Shih, L.Y. Shih, T.H. Sung, Y.C. Chang, Restoration of bone defect and enhancement of bone ingrowth using partially demineralized bone matrix and marrow stromal cells, J. Orthop. Res. 23 (2005) 1293–1299, https://doi.org/10.1016/j.orthres.2005.04.005.1100230609.
[37] R. Quarto, M. Mastrogiacomo, R. Cancedda, S.M. Kutepov, V. Mukhachev, A. Lavroukov, E. Kon, M. Marcacci, Repair of large bone defects with the use of autologous bone marrow stromal cells, N. Engl. J. Med. 344 (2001) 385–386, https://doi.org/10.1056/NEJM200102013440516.
[38] C.M. Cowan, Y.Y. Shi, O.O. Aalami, Y.F. Chou, C. Mari, R. Thomas, N. Quarto, C.H. Contag, B. Wu, M.T. Longaker, Adipose-derived adult stromal cells heal critical-size mouse calvarial defects, Nat. Biotechnol. 22 (2004) 560–567, https://doi.org/10.1038/nbt958.
[39] K. Nishida, M. Yamato, Y. Hayashida, K. Watanabe, K. Yamamoto, E. Adachi, S. Nagai, A. Kikuchi, N. Maeda, H. Watanabe, T. Okano, Y. Tano, Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium, N. Engl. J. Med. 351 (2004) 1187–1196, https://doi.org/10.1056/NEJMoa040455.
[40] T. Shimizu, M. Yamato, A. Kikuchi, T. Okano, Cell sheet engineering for myocardial tissue reconstruction, Biomaterials 24 (2003) 2309–2316, https://doi.org/10.1016/s0142-9612(03)00110-8.
[41] M. Hasegawa, M. Yamato, A. Kikuchi, T. Okano, I. Ishikawa, Human periodontal ligament cell sheets can regenerate periodontal ligament tissue in an athymic rat model, Tissue, Eng. Times 11 (2005) 469–478, https://doi.org/10.1089/ten.2005.11.469.
[42] Y. Shiroyanagi, M. Yamato, Y. Yamazaki, H. Toma, T. Okano, Transplantable urothelial cell sheets harvested noninvasively from temperature-responsive culture surfaces by reducing temperature, Tissue, Eng. Times 9 (2003) 1005–1012, https://doi.org/10.1089/107632703322495646.
[43] H. Ito, Clinical considerations of regenerative medicine in osteoporosis, Curr. Osteoporos. Rep. 12 (2014) 230–234, https://doi.org/10.1007/s11914-014-0201-8.
[44] Z.Y. Zhang, S.H. Teoh, J.H. Hui, N.M. Fisk, M. Choolani, J.K. Chan, The potential of human fetal mesenchymal stem cells for off-the-shelf bone tissue engineering application, Biomaterials 33 (2012) 2656–2672, https://doi.org/10.1016/j.biomaterials.2011.12.025.
[45] K.I. Ko, L.S. Coimbra, C. Tian, J. Alblowi, R.A. Kayal, T.A. Einhorn, L.C. Gerstenfeld, R.J. Pignolo, D.T. Graves, Diabetes reduces mesenchymal stem cells in fracture healing through a TNFα-mediated mechanism, Diabetologia 58 (2015) 633–642, https://doi.org/10.1007/s00125-014-3470-y.
[46] J.H. Lee, Y.K. Lee, S.H. Oh, J. Ahn, Y.E. Lee, J.H. Pyo, Y.Y. Choi, D. Kim, S.C. Bae, Y.K. Sung, D.Y. Kim, A systematic review of diagnostic accuracy of vertebral fracture assessment (VFA) in postmenopausal women and elderly men, Osteoporos. Int. 27 (2016) 1691–1699, https://doi.org/10.1007/s00198-015-3436-z.
[47] R.A. Meyer Jr., P.J. Tsahakis, D.F. Martin, D.M. Banks, M.E. Harrow, G.M. Kiebzak, Age and ovariectomy impair both the normalization of mechanical properties and the accretion of mineral by the fracture callus in rats, J. Orthop. Res. 19 (2001) 428–435, https://doi.org/10.1016/S0736-0266(00)90034-2.
[48] B.D. Sui, C.H. Hu, C.X. Zheng, Y. Jin, Microenvironmental views on mesenchymal stem cell differentiation in aging, J. Dent. Res. 95 (2016) 1333–1340, https://doi.org/10.1177/0022034516653589.
[49] A. Papadopoulou, M. Yiangou, E. Athanasiou, N. Zogas, P. Kaloyannidis, I. Batsis, A. Fassas, A. Anagnostopoulos, E. Yannaki, Mesenchymal stem cells are conditionally therapeutic in preclinical models of rheumatoid arthritis, Ann. Rheum. Dis. 71 (2012) 1733–1740, https://doi.org/10.1136/annrheumdis-2011-200985.
[50] S. Huang, L. Xu, Y. Sun, S. Lin, W. Gu, Y. Liu, J. Zhang, L. Chen, G. Li, Systemic administration of allogeneic mesenchymal stem cells does not halt osteoporotic bone loss in ovariectomized rats, PloS One 11 (2016) e0163131, , https://doi.org/10.1371/journal.pone.0163131.
[51] B.D. Sui, C.H. Hu, C.X. Zheng, Y. Shuai, X.N. He, P.P. Gao, P. Zhao, M. Li, X.Y. Zhang, T. He, K. Xuan, Y. Jin, Recipient glycemic micro-environments govern therapeutic effects of mesenchymal stem cell infusion on osteopenia, Theranostics 7 (2017) 1225–1244, https://doi.org/10.7150/thno.18181.
[52] Y. Liu, L. Wang, T. Kikuiri, K. Akiyama, C. Chen, X. Xu, R. Yang, W. Chen, S. Wang, S. Shi, Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-γ and TNF-α, Nat. Med. 17 (2011) 1594–1601, https://doi.org/10.1038/nm.2542.
[53] S. Liu, D. Liu, C. Chen, K. Hamamura, A. Moshaverinia, R. Yang, Y. Liu, Y. Jin, S. Shi, MSC transplantation improves osteopenia via epigenetic regulation of notch signaling in lupus, Cell Metabol. 22 (2015) 606–618, https://doi.org/10.1016/j.cmet.2015.08.018.
[54] Y. Shuai, L. Liao, X. Su, Y. Yu, B. Shao, H. Jing, X. Zhang, Z. Deng, Y. Jin, Melatonin treatment improves mesenchymal stem cells therapy by preserving stemness during long-term in vitro expansion, Theranostics 6 (2016), https://doi.org/10.7150/thno.15412 1899–1917.
[55] E.A. Jones, A. English, S.E. Kinsey, L. Straszynski, P. Emery, F. Ponchel, D. McGonagle, Optimization of a flow cytometry-based protocol for detection and phenotypic characterization of multipotent mesenchymal stromal cells from human bone marrow, Cytometry. B. Clin. Cytom. 70 (2006) 391–399, https://doi.org/10.1002/cyto.b.20118.
[56] R. Bielby, E. Jones, D. McGonagle, The role of mesenchymal stem cells in maintenance and repair of bone, Injury 38 (2007), https://doi.org/10.1016/j.injury.2007.02.007 S26–S32.
[57] L.G. Raisz, Pathogenesis of osteoporosis: concepts, conflicts, and prospects, J. Clin. Invest. 115 (2005) 3318–3325, https://doi.org/10.1172/JCI27071.
[58] M.C. Walsh, N. Kim, Y. Kadono, J. Rho, S.Y. Lee, J. Lorenzo, Y. Choi, Osteoimmunology: interplay between the immune system and bone metabolism, Annu. Rev. Immunol. 24 (2006) 33–63, https://doi.org/10.1146/annurev.immunol.24.021605.090646.
[59] P.J. Marie, M. Kassem, Osteoblasts in osteoporosis: past, emerging, and future anabolic targets, Eur. J. Endocrinol. 165 (2011) 1–10, https://doi.org/10.1530/EJE-11-0132.
[60] W.J. Boyle, W.S. Simonet, D.L. Lacey, Osteoclast differentiation and activation, Nature 423 (2003) 337–342, https://doi.org/10.1038/nature01658.
[61] B. Sui, C. Hu, Y. Jin, Mitochondrial metabolic failure in telomere attrition-provoked aging of bone marrow mesenchymal stem cells, Biogerontology 17 (2016) 267–279, https://doi.org/10.1007/s10522-015-9609-5.
[62] F.A. Fierro, J.A. Nolta, I.E. Adamopoulos, Concise review: stem cells in osteoimmunology, Stem Cell. 35 (2017) 1461–1467, https://doi.org/10.1002/stem.2625.
[63] A.J. Friedenstein, R.K. Chailakhyan, U.V. Gerasimov, Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers, Cell. Tissue, Kinet 20 (1987) 263–272, https://doi.org/10.1111/j.1365-2184.1987.tb01309.x.
[64] C. Ikebe, K. Suzuki, Mesenchymal stem cells for regenerative therapy: optimization of cell preparation protocols, BioMed Res. Int. 2014 (2014) 951512, https://doi.org/10.1155/2014/951512.
[65] A.J. Friedenstein, R.K. Chailakhjan, K.S. Lalykina, The development of fibroblast colonies in monolayer cultures of Guinea-pig bone marrow and spleen cells, Cell. Tissue, Kinet 3 (1970) 393–403, https://doi.org/10.1111/j.1365-2184.1970.tb00347.x.
[66] B.O. Zhou, R. Yue, M.M. Murphy, J.G. Peyer, S.J. Morrison, Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow, Cell. Stem. Cell. 15 (2014) 154–168, https://doi.org/10.1016/j.stem.2014.06.008.
[67] C.K. Chan, E.Y. Seo, J.Y. Chen, D. Lo, A. McArdle, R. Sinha, R. Tevlin, J. Seita, J. Vincent-Tompkins, T. Wearda, W.J. Lu, K. Senarath-Yapa, M.T. Chung, O. Marecic, M. Tran, K.S. Yan, R. Upton, G.G. Walmsley, A.S. Lee, D. Sahoo, C.J. Kuo, I.L. Weissman, M.T. Longaker, Identification and specification of the mouse skeletal stem cell, Cell 160 (2015) 285–298, https://doi.org/10.1016/j.cell.2014.12.002.
[68] C. Chen, D. Wang, A. Moshaverinia, D. Liu, X. Kou, W. Yu, R. Yang, L. Sun, S. Shi, Mesenchymal stem cell transplantation in tight-skin mice identifies miR-151-5p as a therapeutic target for systemic sclerosis, Cell Res. 27 (2017) 559–577, https://doi.org/10.1038/cr.2017.11.
[69] J. Goldhahn, D. Little, P. Mitchell, N.L. Fazzalari, I.R. Reid, P. Aspenberg, D. Marsh, ISFR working group drugs and fracture repair, Evidence for anti-osteoporosis therapy in acute fracture situations–recommendations of a multidisciplinary workshop of the International Society for Fracture Repair, Bone 46 (2010) 267–271, https://doi.org/10.1016/j.bone.2009.10.004.
[70] L. Liao, X. Yang, X. Su, C. Hu, X. Zhu, N. Yang, X. Chen, S. Shi, S. Shi, Y. Jin, Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow, Cell Death Dis. 4 (2013) e600, , https://doi.org/10.1038/cddis.2013.130.
[71] B. Shao, L. Liao, Y. Yu, Y. Shuai, X. Su, H. Jing, D. Yang, Y. Jin, Estrogen preserves Fas ligand levels by inhibiting microRNA-181a in bone marrow-derived mesenchymal stem cells to maintain bone remodeling balance, Faseb. J. 29 (2015) 3935–3944, https://doi.org/10.1096/fj.15-272823.
[72] B. Sui, C. Hu, L. Liao, Y. Chen, X. Zhang, X. Fu, C. Zheng, M. Li, L. Wu, X. Zhao, Y. Jin, Mesenchymal progenitors in osteopenias of diverse pathologies: differential characteristics in the common shift from osteoblastogenesis to adipogenesis, Sci. Rep. 6 (2016) 30186, https://doi.org/10.1038/srep30186.
[73] Y. Liu, X. Kou, C. Chen, W. Yu, Y. Su, Y. Kim, S. Shi, Y. Liu, Chronic high dose alcohol induces osteopenia via activation of mTOR signaling in bone marrow mesenchymal stem cells, Stem Cell. 34 (2016) 2157–2168, https://doi.org/10.1002/stem.2392.
[74] A. Uccelli, L. Moretta, V. Pistoia, Mesenchymal stem cells in health and disease, Nat. Rev. Immunol. 8 (2008) 726–736, https://doi.org/10.1038/nri2395.
[75] S. Naik, S.B. Larsen, C.J. Cowley, E. Fuchs, Two to tango: dialog between immunity and stem cells in health and disease, Cell 175 (2018) 908–920, https://doi.org/10.1016/j.cell.2018.08.071.
[76] J. Fujisaki, J. Wu, A.L. Carlson, L. Silberstein, P. Putheti, R. Larocca, W. Gao, T.I. Saito, C. Lo Celso, H. Tsuyuzaki, T. Sato, D. Côté, M. Sykes, T.B. Strom, D.T. Scadden, C.P. Lin, In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche, Nature 474 (2011) 216–219, https://doi.org/10.1038/nature10160.
[77] Y. Hirata, K. Furuhashi, H. Ishii, H.W. Li, S. Pinho, L. Ding, S.C. Robson, P.S. Frenette, J. Fujisaki, CD150high bone marrow Tregs maintain hematopoietic stem cell quiescence and immune privilege via adenosine, Cell. Stem. Cell. 22 (2018) 445–453, https://doi.org/10.1016/j.stem.2018.01.017.
[78] M. Gersemann, E.F. Stange, J. Wehkamp, From intestinal stem cells to inflammatory bowel diseases, World J. Gastroenterol. 17 (2011) 3198–3203, https://doi.org/10.3748/wjg.v17.i27.3198.
[79] M.A. Lowes, A.M. Bowcock, J.G. Krueger, Pathogenesis and therapy of psoriasis, Nature 445 (2007) 866–873, https://doi.org/10.1038/nature05663.
[80] S. Naik, S.B. Larsen, N.C. Gomez, K. Alaverdyan, A. Sendoel, S. Yuan, L. Polak, A. Kulukian, S. Chai, E. Fuchs, Inflammatory memory sensitizes skin epithelial stem cells to tissue damage, Nature 550 (2017) 475–480, https://doi.org/10.1038/nature24271.
[81] J. Ordovas-Montanes, D.F. Dwyer, S.K. Nyquist, K.M. Buchheit, M. Vukovic, C. Deb, M.H. Wadsworth 2nd, T.K. Hughes, S.W. Kazer, E. Yoshimoto, K.N. Cahill,

N. Bhattacharyya, H.R. Katz, B. Berger, T.M. Laidlaw, J.A. Boyce, N.A. Barrett, A.K. Shalek, Allergic inflammatory memory in human respiratory epithelial progenitor cells, Nature 560 (2018) 649–654, https://doi.org/10.1038/s41586-018-0449-8.
[82] C.A. Lindemans, M. Calafiore, A.M. Mertelsmann, M.H. O'Connor, J.A. Dudakov, R.R. Jenq, E. Velardi, L.F. Young, O.M. Smith, G. Lawrence, J.A. Ivanov, Y.Y. Fu, S. Takashima, G. Hua, M.L. Martin, K.P. O'Rourke, Y.H. Lo, M. Mokry, M. Romera-Hernandez, T. Cupedo, L. Dow, E.E. Nieuwenhuis, N.F. Shroyer, C. Liu, R. Kolesnick, M.R.M. van den Brink, A.M. Hanash, Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration, Nature 528 (2015) 560–564, https://doi.org/10.1038/nature16460.
[83] J. von Moltke, M. Ji, H.E. Liang, R.M. Locksley, Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit, Nature 529 (2016) 221–225, https://doi.org/10.1038/nature16161.
[84] M. Casanova-Acebes, C. Pitaval, L.A. Weiss, C. Nombela-Arrieta, R. Chèvre, N. A-González, Y. Kunisaki, D. Zhang, N. van Rooijen, L.E. Silberstein, C. Weber, T. Nagasawa, P.S. Frenette, A. Castrillo, A. Hidalgo, Rhythmic modulation of the hematopoietic niche through neutrophil clearance, Cell 153 (2013) 1025–1035, https://doi.org/10.1016/j.cell.2013.04.040.
[85] E. Bowers, A. Slaughter, P.S. Frenette, R. Kuick, O.M. Pello, D. Lucas, Granulocyte-derived TNFα promotes vascular and hematopoietic regeneration in the bone marrow, Nat. Med. 24 (2018) 95–102, https://doi.org/10.1038/nm.4448.
[86] R. Kane, P.X. Ma1, Mimicking the nanostructure of bone matrix to regenerate bone, Mater. Today 16 (2013) 418–423, https://doi.org/10.1016/j.mattod.2013.11.001.
[87] E. Rosenberg, L.F. Rose, Biologic and clinical considerations for autografts and allografts in periodontal regeneration therapy, Dent, Clin. North. Am. 42 (1998) 467–490.
[88] M.A. Reynolds, M.E. Aichelmann-Reidy, G.L. Branch-Mays, Regeneration of periodontal tissue: bone replacement grafts, Dent, Clin. North. Am. 54 (2010) 55–71, https://doi.org/10.1016/j.cden.2009.09.003.
[89] S.N. Khan, F.P. Jr Cammisa, H.S. Sandhu, A.D. Diwan, F.P. Girardi, J.M. Lane, The biology of bone grafting, J. Am. Acad. Orthop. Surg. 13 (2005) 77–86.
[90] M.K. Sen, T. Miclau, Autologous iliac crest bone graft: should it still be the gold standard for treating nonunions? Injury 38 (2007), https://doi.org/10.1016/j.injury.2007.02.012 S75–S80.
[91] A. Torroni, Engineered bone grafts and bone flaps for maxillofacial defects: state of the art, J. Oral Maxillofac. Surg. 67 (2009) 1121–1127, https://doi.org/10.1016/j.joms.2008.11.020.
[92] K. Doi, S. Tominaga, T. Shibata, Bone grafts with microvascular anastomoses of vascular pedicles: an experimental study in dogs, J. Bone. Joint. Surg. Am. 59 (1977) 809–815.
[93] P.C. Dell, H. Burchardt, F.P. Jr Glowczewskie, A roentgenographic, biomechanical, and histological evaluation of vascularized and non-vascularized segmental fibular canine autografts, J. Bone. Joint. Surg. Am. 67 (1985) 105–112.
[94] U. Kneser, D.J. Schaefer, E. Polykandriotis, R.E. Horch, Tissue engineering of bone: the reconstructive surgeon's point of view, J. Cell Mol. Med. 10 (2006) 7–19, https://doi.org/10.1111/j.1582-4934.2006.tb00287.x.
[95] C. Sbordone, L. Sbordone, P. Toti, R. Martuscelli, L. Califano, F. Guidetti, Volume changes of grafted autogenous bone in sinus augmentation procedure, J. Oral Maxillofac. Surg. 69 (2011) 1633–1641, https://doi.org/10.1016/j.joms.2010.12.004.
[96] P. Habibovic, K. de Groot, Osteoinductive biomaterials–properties and relevance in bone repair, J. Tissue. Eng. Regen. Med. 1 (2007) 25–32, https://doi.org/10.1002/term.5.
[97] B. Baroli, From natural bone grafts to tissue engineering therapeutics: brainstorming on pharmaceutical formulative requirements and challenges, J. Pharmaceut. Sci. 98 (2009) 1317–1375, https://doi.org/10.1002/jps.21528.
[98] B.E. Buck, T.I. Malinin, Human bone and tissue allografts. Preparation and safety, Clin. Orthop. Relat. Res. (1994) 8–17.
[99] D. Chow, M.L. Nunalee, D.W. Lim, A.J. Simnick, A. Chilkoti, Peptide-based biopolymers in biomedicine and biotechnology, Mater. Sci. Eng. R Rep. 62 (2008) 125–155, https://doi.org/10.1016/j.mser.2008.04.004.
[100] F.Z. Cui, Y. Li, J. Ge, Self-assembly of mineralized collagen composites, Mater. Sci. Eng. R Rep. 57 (2007) 1–27.
[101] J.S. Hanker, B.L. Giammara, Biomaterials and biomedical devices, Science 242 (1988) 885–892, https://doi.org/10.1126/science.3055300.
[102] E.S. Place, N.D. Evans, M.M. Stevens, Complexity in biomaterials for tissue engineering, Nat. Mater. 8 (2009) 457–470, https://doi.org/10.1038/nmat2441.
[103] D.F. Williams, On the nature of biomaterials, Biomaterials 30 (2009) 5897–5909, https://doi.org/10.1016/j.biomaterials.2009.07.027.
[104] D.F. Williams, The biomaterials conundrum in tissue engineering, Tissue, Eng. Part. A. 20 (2014) 1129–1131, https://doi.org/10.1089/ten.tea.2013.0769.
[105] F.M. Chen, L.A. Wu, M. Zhang, R. Zhang, H.H. Sun, Homing of endogenous stem/progenitor cells for in situ tissue regeneration: promises, strategies, and translational perspectives, Biomaterials 32 (2011) 3189–3209, https://doi.org/10.1016/j.biomaterials.2010.12.032.
[106] K.B. Fonseca, P.L. Granja, C.C. Barrias, Engineering proteolytically degradable artifificial extracellular matrices, Prog. Polym. Sci. 39 (2014) 2010–2029.
[107] B.S. Kim, I.K. Park, T. Hoshiba, H.L. Jiang, Y.J. Choi, T. Akaike, C.S. Cho, Design of artifificial extracellular matrices for tissue engineering, Prog. Polym. Sci. 36 (2011) 238–268.
[108] S.F. Badylak, D.J. Weiss, A. Caplan, P. Macchiarini, Engineered whole organs and complex tissues, Lancet 379 (2012) 943–952, https://doi.org/10.1016/S0140-6736(12)60073-7.
[109] S.F. Badylak, The extracellular matrix as a biologic scaffold material, Biomaterials 28 (2007) 3587–3593, https://doi.org/10.1016/j.biomaterials.2007.04.043.
[110] B.N. Brown, S.F. Badylak, Extracellular matrix as an inductive scaffold for functional tissue reconstruction, Transl. Res. 163 (2014) 268–285, https://doi.org/10.1016/j.trsl.2013.11.003.
[111] J.A. Hubbell, Materials as morphogenetic guides in tissue engineering, Curr. Opin. Biotechnol. 14 (2003) 551–558, https://doi.org/10.1016/j.copbio.2003.09.004.
[112] Y. Sun, Y. Jiang, Q. Liu, T. Gao, J.Q. Feng, P. Dechow, R.N. D'Souza, C. Qin, X. Liu, Biomimetic engineering of nanofibrous gelatin scaffolds with noncollagenous proteins for enhanced bone regeneration, Tissue, Eng. Part. A. 19 (2013) 1754–1763, https://doi.org/10.1089/ten.TEA.2012.0567.
[113] P. Huang, X. Bi, J. Gao, L. Sun, S. Wang, S. Chen, X. Fan, Z. You, Y. Wang, Phosphorylated poly (sebacoyl diglyceride) - a phosphate functionalized biodegradable polymer for bone tissue engineering, J. Mater. Chem. B. 4 (2016) 2090–2101, https://doi.org/10.1039/c5tb02542g.
[114] W. Wei, J. Li, S. Chen, M. Chen, Q. Xie, H. Sun, J. Ruan, H. Zhou, X. Bi, A. Zhuang, Z. You, P. Gu, X. Fan, In vitro osteogenic induction of bone marrow mesenchymal stem cells with a decellularized matrix derived from human adipose stem cells and in vivo implantation for bone regeneration, J. Mater. Chem. B. 5 (2017) 2468–2482, https://doi.org/10.1039/c6tb03150a.
[115] J.D. Kretlow, S. Young, L. Klouda, M. Wong, A.G. Mikos, Injectable biomaterials for regenerating complex craniofacial tissues, Adv. Mater. 21 (2009) 3368–3393, https://doi.org/10.1002/adma.200802009.
[116] H.S. Sandhu, S.N. Khan, D.Y. Suh, S.D. Boden, Demineralized bone matrix, bone morphogenetic proteins, and animal models of spine fusion: an overview, Eur. Spine J. 10 (2001), https://doi.org/10.1007/s005860100303 S122–S131.
[117] T. Boyce, J. Edwards, N. Scarborough, Allograft bone. The influence of processing on safety and performance, Orthop. Clin. N. Am. 30 (1999) 571–581, https://doi.org/10.1016/s0030-5898(05)70110-3.
[118] C.W. Cheng, L.D. Solorio, E. Alsberg, Decellularized tissue and cell-derived extracellular matrices as scaffolds for orthopaedic tissue engineering, Biotechnol. Adv. 32 (2014) 462–484, https://doi.org/10.1016/j.biotechadv.2013.12.012.
[119] F.M. Chen, X. Liu, Advancing biomaterials of human origin for tissue engineering, Prog. Polym. Sci. 53 (2016) 86–168, https://doi.org/10.1016/j.progpolymsci.2015.02.004.
[120] M.P. Bostrom, X. Yang, M. Kennan, H. Sandhu, E. Dicarlo, J.M. Lane, An unexpected outcome during testing of commercially available demineralized bone graft materials: how safe are the nonallograft components? Spine 26 (2001) 1425–1428, https://doi.org/10.1097/00007632-200107010-00007.
[121] D.C. Markel, S.T. Guthrie, B. Wu, Z. Song, P.H. Wooley, Characterization of the inflammatory response to four commercial bone graft substitutes using a murine biocompatibility model, J. Inflamm. Res. 5 (2012) 13–18, https://doi.org/10.2147/JIR.S21411.
[122] B. Wildemann, A. Kadow-Romacker, N.P. Haas, G. Schmidmaier, Quantification of various growth factors in different demineralized bone matrix preparations, J. Biomed. Mater. Res. A. 81 (2007) 437–442, https://doi.org/10.1002/jbm.a.31085.
[123] M.S. Lee, D.H. Lee, J. Jin, G. Tae, Y.M. Shin, H.S. Yang, Biofabrication and application of decellularized bone extracellular matrix for effective bone regeneration, J. Ind. Eng. Chem. 83 (2019), https://doi.org/10.1016/j.jiec.2019.12.005 323–322.
[124] National Institutes of Health, A Service of the U.S. https://www.clinicaltrials.gov/.
[125] American Society of Gene and Cell Therapy, (2017) http://www.asgct.org/general-public/educational-resources/gene-therapy–and-cell-therapy-defined accessed 7 Aug 2017) National Institutes of Health, A service of the U.Shttps://www.clinicaltrials.gov/.
[126] B. Parekkadan, J.M. Milwid, Mesenchymal stem cells as therapeutics, Annu. Rev. Biomed. Eng. 12 (2010) 87–117, https://doi.org/10.1146/annurev-bioeng-070909-105309.
[127] A.I. Caplan, D. Correa, The MSC: an injury drugstore, Cell. Stem. Cell. 9 (2011) 11–15, https://doi.org/10.1016/j.stem.2011.06.008.
[128] M.M. Lalu, L. McIntyre, C. Pugliese, D. Fergusson, B.W. Winston, J.C. Marshall, J. Granton, D.J. Stewart, Canadian Critical Care Trials Group, Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials, PLoS. One. 7 (2012) e47559, , https://doi.org/10.1371/journal.pone.0047559.
[129] M. Guan, W. Yao, R. Liu, K.S. Lam, J. Nolta, J. Jia, B. Panganiban, L. Meng, P. Zhou, M. Shahnazari, R.O. Ritchie, N.E. Lane, Directing mesenchymal stem cells to bone to augment bone formation and increase bone mass, Nat. Med. 18 (2012) 456–462, https://doi.org/10.1038/nm.2665.
[130] W. Yao, Y.E. Lay, A. Kot, R. Liu, H. Zhang, H. Chen, K. Lam, N.E. Lane, Improved mobilization of exogenous mesenchymal stem cells to bone for fracture healing and sex difference, Stem Cell. 34 (2016) 2587–2600, https://doi.org/10.1002/stem.2433.
[131] A.M. Nair, Y.T. Tsai, K.M. Shah, J. Shen, H. Weng, J. Zhou, X. Sun, R. Saxena Jr., J. Borrelli, L. Tang, The effect of erythropoietin on autologous stem cell-mediated bone regeneration, Biomaterials 34 (2013) 7364–7371, https://doi.org/10.1016/j.biomaterials.2013.06.031.
[132] P. Chen, J. Tao, S. Zhu, Y. Cai, Q. Mao, D. Yu, J. Dai, H. Ouyang, Radially oriented collagen scaffold with SDF-1 promotes osteochondral repair by facilitating cell homing, Biomaterials 39 (2015) 114–123, https://doi.org/10.1016/j.biomaterials.2014.10.049.
[133] C.H. Lee, J.L. Cook, A. Mendelson, E.K. Moioli, H. Yao, J.J. Mao, Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study, Lancet 376 (2010) 440–448, https://doi.org/10.1016/S0140-6736(10)60668-X.
[134] C.H. Lee, B. Shah, E.K. Moioli, J.J. Mao, CTGF directs fibroblast differentiation

from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model, J. Clin. Invest. 120 (2010) 3340–3349, https://doi.org/10.1172/JCI43230.
[135] Y. Wu, L. Cao, L. Xia, Q. Wu, J. Wang, X. Wang, L. Xu, Y. Zhou, Y. Xu, X. Jiang, Evaluation of osteogenesis and angiogenesis of icariin in local controlled release and systemic delivery for calvarial defect in ovariectomized rats, Sci. Rep. 7 (2017) 5077, https://doi.org/10.1038/s41598-017-05392-z.
[136] T. Ono, H. Takayanagi, Osteoimmunology in bone fracture healing, Curr. Osteoporos. Rep. 15 (2017) 367–375, https://doi.org/10.1007/s11914-017-0381-0.
[137] C. Chen, K. Akiyama, D. Wang, X. Xu, B. Li, A. Moshaverinia, F. Brombacher, L. Sun, S. Shi, mTOR inhibition rescues osteopenia in mice with systemic sclerosis, J. Exp. Med. 212 (2015) 73–91, https://doi.org/10.1084/jem.20140643.
[138] S.M. Abdelmagid, M.F. Barbe, F.F. Safadi, Role of inflammation in the aging bones, Life Sci. 123 (2015) 25–34, https://doi.org/10.1016/j.lfs.2014.11.011.
[139] L. Wang, Y. Zhao, Y. Liu, K. Akiyama, C. Chen, C. Qu, Y. Jin, S. Shi, IFN-γ and TNF-α synergistically induce mesenchymal stem cell impairment and tumorigenesis via NFκB signaling, Stem Cell. 31 (2013) 1383–1395, https://doi.org/10.1002/stem.1388.
[140] C. Li, B. Li, Z. Dong, L. Gao, X. He, L. Liao, C. Hu, Q. Wang, Y. Jin, Lipopolysaccharide differentially affects the osteogenic differentiation of periodontal ligament stem cells and bone marrow mesenchymal stem cells through Toll-like receptor 4 mediated nuclear factor κB pathway, Stem Cell Res. Ther. 5 (2014) 67, https://doi.org/10.1186/scrt456.
[141] P. Xue, B. Li, Y. An, J. Sun, X. He, R. Hou, G. Dong, D. Fei, F. Jin, Q. Wang, Y. Jin, Decreased MORF leads to prolonged endoplasmic reticulum stress in periodontitis-associated chronic inflammation, Cell Death Differ. 23 (2016) 1862–1872, https://doi.org/10.1038/cdd.2016.74.
[142] S. Geissler, M. Textor, K. Schmidt-Bleek, O. Klein, M. Thiele, A. Ellinghaus, D. Jacobi, A. Ode, C. Perka, A. Dienelt, J. Klose, G. Kasper, G.N. Duda, P. Strube, In serum veritas-in serum sanitas? Cell non-autonomous aging compromises differentiation and survival of mesenchymal stromal cells via the oxidative stress pathway, Cell Death Dis. 4 (2013) e970, , https://doi.org/10.1038/cddis.2013.501.
[143] L. Ming, F. Jin, P. Huang, H. Luo, W. Liu, L. Zhang, W. Yuan, Y. Zhang, Y. Jin, Licochalcone A up-regulates of FasL in mesenchymal stem cells to strengthen bone formation and increase bone mass, Sci. Rep. 4 (2014) 7209, https://doi.org/10.1038/srep07209.
[144] H. Jing, L. Liao, Y. An, X. Su, S. Liu, Y. Shuai, X. Zhang, Y. Jin, Suppression of EZH2 prevents the shift of osteoporotic MSC fate to adipocyte and enhances bone formation during osteoporosis, Mol. Ther. 24 (2016) 217–229, https://doi.org/10.1038/mt.2015.152.
[145] L. Lv, W. Ge, Y. Liu, G. Lai, H. Liu, W. Li, Y. Zhou, Lysine-specific demethylase 1 inhibitor rescues the osteogenic ability of mesenchymal stem cells under osteoporotic conditions by modulating H3K4 methylation, Bone. Res. 4 (2016) 16037, https://doi.org/10.1038/boneres.2016.37.
[146] C. Wang, J. Wang, J. Li, G. Hu, S. Shan, Q. Li, X. Zhang, KDM5A controls bone morphogenic protein 2-induced osteogenic differentiation of bone mesenchymal stem cells during osteoporosis, Cell Death Dis. 7 (2016) e2335, https://doi.org/10.1038/cddis.2016.238.
[147] C.J. Li, P. Cheng, M.K. Liang, Y.S. Chen, Q. Lu, J.Y. Wang, Z.Y. Xia, H.D. Zhou, X. Cao, H. Xie, E.Y. Liao, X.H. Luo, MicroRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation, J. Clin. Invest. 125 (2015) 1509–1522, https://doi.org/10.1172/JCI77716.
[148] X. Zhang, Y. Li, Y.E. Chen, J. Chen, P.X. Ma, Cell-free 3D scaffold with two-stage delivery of miRNA-26a to regenerate critical-sized bone defects, Nat. Commun. 7 (2016) 10376, https://doi.org/10.1038/ncomms10376.
[149] M. Yoshizuka, T. Nakasa, Y. Kawanishi, S. Hachisuka, T. Furuta, S. Miyaki, N. Adachi, M. Ochi, Inhibition of microRNA-222 expression accelerates bone healing with enhancement of osteogenesis, chondrogenesis, and angiogenesis in a rat refractory fracture model, J. Orthop. Sci. 21 (2016) 852–858, https://doi.org/10.1016/j.jos.2016.07.021.
[150] Y. Deng, X. Bi, H. Zhou, Z. You, Y. Wang, P. Gu, X. Fan, Repair of critical-sized bone defects with anti-miR-31-expressing bone marrow stromal stem cells and poly (glycerol sebacate) scaffolds, Eur. Cell. Mater. 27 (2014) 13–25, https://doi.org/10.22203/ecm.v027a02.
[151] N. Yang, G. Wang, C. Hu, Y. Shi, L. Liao, S. Shi, Y. Cai, S. Cheng, X. Wang, Y. Liu, L. Tang, Y. Ding, Y. Jin, Tumor necrosis factor α suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis, J. Bone Miner. Res. 28 (2013) 559–573, https://doi.org/10.1002/jbmr.1798.
[152] T. Yamaza, Y. Miura, Y. Bi, Y. Liu, K. Akiyama, W. Sonoyama, V. Patel, S. Gutkind, M. Young, S. Gronthos, A. Le, C.Y. Wang, W. Chen, S. Shi, Pharmacologic stem cell based intervention as a new approach to osteoporosis treatment in rodents, PLoS. One. 3 (2008) e2615, , https://doi.org/10.1371/journal.pone.0002615.
[153] B. Sui, C. Hu, X. Zhang, P. Zhao, T. He, C. Zhou, X. Qiu, N. Chen, X. Zhao, Y. Jin, Allogeneic mesenchymal stem cell therapy promotes osteoblastogenesis and prevents glucocorticoid-induced osteoporosis, Stem. Cells. Transl. Med. 5 (2016) 1238–1246, https://doi.org/10.5966/sctm.2015-0347.
[154] Y. Miura, Z. Gao, M. Miura, B.M. Seo, W. Sonoyama, W. Chen, S. Gronthos, L. Zhang, S. Shi, Mesenchymal stem cell-organized bone marrow elements: an alternative hematopoietic progenitor resource, Stem Cell. 24 (2006) 2428–2436, https://doi.org/10.1634/stemcells.2006-0089.
[155] K. Akiyama, C. Chen, D. Wang, X. Xu, C. Qu, T. Yamaza, T. Cai, W. Chen, L. Sun, S. Shi, Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis, Cell. Stem. Cell. 10 (2012) 544–555, https://doi.org/10.1016/j.stem.2012.03.007.
[156] Y. Liu, L. Wang, S. Liu, D. Liu, C. Chen, X. Xu, X. Chen, S. Shi, Transplantation of SHED prevents bone loss in the early phase of ovariectomy-induced osteoporosis, J. Dent. Res. 93 (2014) 1124–1132, https://doi.org/10.1177/0022034514552675.
[157] F. Ezquer, M. Ezquer, D. Contador, M. Ricca, V. Simon, P. Conget, The antidiabetic effect of mesenchymal stem cells is unrelated to their transdifferentiation potential but to their capability to restore Th1/Th2 balance and to modify the pancreatic microenvironment, Stem Cell. 30 (2012) 1664–1674, https://doi.org/10.1002/stem.1132.
[158] Z. Xie, H. Hao, C. Tong, Y. Cheng, J. Liu, Y. Pang, Y. Si, Y. Guo, L. Zang, Y. Mu, W. Han, Human umbilical cord-derived mesenchymal stem cells elicit macrophages into an anti-inflammatory phenotype to alleviate insulin resistance in type 2 diabetic rats, Stem Cell. 34 (2016) 627–639, https://doi.org/10.1002/stem.2238.
[159] P. Bianco, P.G. Robey, Stem cells in tissue engineering, Nature 414 (2001) 118–121, https://doi.org/10.1038/35102181.
[160] K.C. Kemp, J. Hows, C. Donaldson, Bone marrow-derived mesenchymal stem cells, Leuk. Lymphoma 46 (2005) 1531–1544, https://doi.org/10.1080/10428190500215076.
[161] D. Minteer, K.G. Marra, J.P. Rubin, Adipose-derived mesenchymal stem cells: biology and potential applications, Adv. Biochem. Eng. Biotechnol. 129 (2013) 59–71, https://doi.org/10.1007/10_2012_146.
[162] L. Zhao, E.F. Burguera, H.H. Xu, N. Amin, H. Ryou, D.D. Arola, Fatigue and human umbilical cord stem cell seeding characteristics of calcium phosphate-chitosan-biodegradable fiber scaffolds, Biomaterials 31 (2010) 840–847, https://doi.org/10.1016/j.biomaterials.2009.09.106.
[163] A. Can, S. Karahuseyinoglu, Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells, Stem, Cells 25 (2007) 2886–2895, https://doi.org/10.1634/stemcells.2007-0417.
[164] A.R. Sanz, F.S. Carrión, A.P. Chaparro, Mesenchymal stem cells from the oral cavity and their potential value in tissue engineering, Periodontol 67 (2000) 251–267, https://doi.org/10.1111/prd.12070 2015.
[165] C.M. Sedgley, T.M. Botero, Dental stem cells and their sources, Dent. Clin. 56 (2012) 549–561, https://doi.org/10.1016/j.cden.2012.05.004.
[166] J.I. Dawson, J. Kanczler, R. Tare, M. Kassem, R.O. Oreffo, Concise review: bridging the gap: bone regeneration using skeletal stem cell-based strategies - where are we now? Stem Cell. 32 (2014) 35–44, https://doi.org/10.1002/stem.1559.
[167] N.G. Singer, A.I. Caplan, Mesenchymal stem cells: mechanisms of inflammation, Annu. Rev. Pathol. 6 (2011) 457–478, https://doi.org/10.1146/annurev-pathol-011110-130230.
[168] Y. Shi, G. Hu, J. Su, W. Li, Q. Chen, P. Shou, C. Xu, X. Chen, Y. Huang, Z. Zhu, X. Huang, X. Han, N. Xie, G. Ren, Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair, Cell Res. 20 (2010) 510–518, https://doi.org/10.1038/cr.2010.44.
[169] J.M. Hare, J.E. Fishman, G. Gerstenblith, D.L. DiFede Velazquez, J.P. Zambrano, V.Y. Suncion, M. Tracy, E. Ghersin, P.V. Johnston, J.A. Brinker, E. Breton, J. Davis-Sproul, I.H. Schulman, J. Byrnes, A.M. Mendizabal, M.H. Lowery, D. Rouy, P. Altman, C. Wong Po Foo, P. Ruiz, A. Amador, J. Da Silva, I.K. McNiece, A.W. Heldman, R. George, A. Lardo, Comparison of allogeneic vs autologous bone marrow–derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial, J. Am. Med. Assoc. 308 (2012) 2369–2379, https://doi.org/10.1001/jama.2012.25321.
[170] K. Le Blanc, I. Rasmusson, B. Sundberg, C. Götherström, M. Hassan, M. Uzunel, O. Ringdén, Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells, Lancet 363 (2004) 1439–1441, https://doi.org/10.1016/S0140-6736(04)16104-7.
[171] R.H. Lee, M.J. Seo, R.L. Reger, J.L. Spees, A.A. Pulin, S.D. Olson, D.J. Prockop, Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 17438–17443, https://doi.org/10.1073/pnas.0608249103.
[172] B.A. Ashton, T.D. Allen, C.R. Howlett, C.C. Eaglesom, A. Hattori, M. Owen, Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo, Clin. Orthop. Relat. Res. (1980) 294–307.
[173] Y. Yoshida, H. Matsubara, X. Fang, K. Hayashi, I. Nomura, S. Ugaji, T. Hamada, H. Tsuchiya, Adipose-derived stem cell sheets accelerate bone healing in rat femoral defects, PloS One 14 (2019) e0214488, , https://doi.org/10.1371/journal.pone.0214488.
[174] A.M. Parker, A.J. Katz, Adipose-derived stem cells for the regeneration of damaged tissues, Expet Opin. Biol. Ther. 6 (2006) 567–578, https://doi.org/10.1517/14712598.6.6.567.
[175] P.A. Zuk, M. Zhu, H. Mizuno, J. Huang, J.W. Futrell, A.J. Katz, P. Benhaim, H.P. Lorenz, M.H. Hedrick, Multilineage cells from human adipose tissue: implications for cell-based therapies, Tissue Eng. 7 (2) (2001) 211–228, https://doi.org/10.1089/107632701300062859.
[176] J.M. Murphy, D.J. Fink, E.B. Hunziker, F.P. Barry, Stem cell therapy in a caprine model of osteoarthritis, Arthritis Rheum. 48 (2003) 3464–3474, https://doi.org/10.1002/art.11365.
[177] R. Hass, C. Kasper, S. Böhm, R. Jacobs, Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC, Cell Commun. Signal. 9 (2011) 12, https://doi.org/10.1186/1478-811X-9-12.
[178] Y. Zhu, T. Liu, K. Song, X. Fan, X. Ma, Z. Cui, Adipose-derived stem cell: a better stem cell than BMSC, Cell Biochem. Funct. 26 (2008) 664–675, https://doi.org/10.1002/cbf.1488.
[179] A. Mirsaidi, K.N. Kleinhans, M. Rimann, A.N. Tiaden, M. Stauber, K.L. Rudolph,

P.J. Richards, Telomere length, telomerase activity and osteogenic differentiation are maintained in adipose-derived stromal cells from senile osteoporotic SAMP6 mice, J. Tissue. Eng. Regen. Med. 6 (2012) 378–390, https://doi.org/10.1002/term.440.
[180] K.R. McIntosh, T. Frazier, B.G. Rowan, J.M. Gimble, Evolution and future prospects of adipose-derived immunomodulatory cell therapeutics, Expet Rev. Clin. Immunol. 9 (2013) 175–184, https://doi.org/10.1586/eci.12.96.
[181] X. Ye, P. Zhang, S. Xue, Y. Xu, J. Tan, G. Liu, Adipose-derived stem cells alleviate osteoporosis by enhancing osteogenesis and inhibiting adipogenesis in a rabbit model, Cytotherapy 16 (2014) 1643–1655, https://doi.org/10.1016/j.jcyt.2014.07.009.
[182] P. Ercal, G.G. Pekozer, G.T. Kose, Dental stem cells in bone tissue engineering: current overview and challenges, Adv. Exp. Med. Biol. 1107 (2018) 113–127, https://doi.org/10.1007/5584_2018_171.
[183] G.T. Huang, S. Gronthos, S. Shi, Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine, J. Dent. Res. 88 (2009) 792–806, https://doi.org/10.1177/0022034509340867.
[184] M.E. Yalvac, M. Ramazanoglu, A.A. Rizvanov, F. Sahin, O.F. Bayrak, U. Salli, A. Palotás, G.T. Kose, Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neo-vascularization, osteo-, adipo- and neurogenesis, Pharmacogenomics J. 10 (2010) 105–113, https://doi.org/10.1038/tpj.2009.40.
[185] S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi, Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 13625–13630, https://doi.org/10.1073/pnas.240309797.
[186] S. Shi, P.G. Robey, S. Gronthos, Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis, Bone 29 (2001) 532–539, https://doi.org/10.1016/s8756-3282(01)00612-3.
[187] S. Gronthos, J. Brahim, W. Li, L.W. Fisher, N. Cherman, A. Boyde, P. DenBesten, P.G. Robey, S. Shi, Stem cell properties of human dental pulp stem cells, J. Dent. Res. 81 (2002) 531–535, https://doi.org/10.1177/154405910208100806.
[188] N. Wada, D. Menicanin, S. Shi, P.M. Bartold, S. Gronthos, Immunomodulatory properties of human periodontal ligament stem cells, J. Cell. Physiol. 219 (2009) 667–676, https://doi.org/10.1002/jcp.21710.
[189] Z. Kadkhoda, A. Safarpour, F. Azmoodeh, S. Adibi, A. Khoshzaban, N. Bahrami, Histopathological comparison between bone marrow- and periodontium-derived stem cells for bone regeneration in rabbit calvaria, Int. J. Organ. Transplant. Med. 7 (2016) 9–18.
[190] G.B. Tomar, R.K. Srivastava, N. Gupta, A.P. Barhanpurkar, S.T. Pote, H.M. Jhaveri, G.C. Mishra, M.R. Wani, Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine, Biochem. Biophys. Res. Commun. 393 (2010) 377–383, https://doi.org/10.1016/j.bbrc.2010.01.126.
[191] Q. Zhang, S. Shi, Y. Liu, J. Uyanne, Y. Shi, S. Shi, A.D. Le, Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis, J. Immunol. 183 (2009) 7787–7798, https://doi.org/10.4049/jimmunol.0902318.
[192] X. Xu, C. Chen, K. Akiyama, Y. Chai, A.D. Le, Z. Wang, S. Shi, Gingivae contain neural-crest- and mesoderm-derived mesenchymal stem cells, J. Dent. Res. 92 (2013) 825–832, https://doi.org/10.1177/0022034513497961.
[193] Q.Z. Zhang, W.R. Su, S.H. Shi, P. Wilder-Smith, A.P. Xiang, A. Wong, A.L. Nguyen, C.W. Kwon, A.D. Le, Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing, Stem Cell. 28 (2010) 1856–1868, https://doi.org/10.1002/stem.503.
[194] F. Wang, M. Yu, X. Yan, Y. Wen, Q. Zeng, W. Yue, P. Yang, X. Pei, Gingiva-derived mesenchymal stem cell-mediated therapeutic approach for bone tissue regeneration, Stem Cell. Dev. 20 (2011) 2093–2102, https://doi.org/10.1089/scd.2010.0523.
[195] Sultan Alghaithi, Gingival MSCs Improve Bone Phenotype in Ovariectomy-Induced Osteoporosis via Programmed Cell Death Pathway vol. 49, (2019) Dental Theses https://repository.upenn.edu/dental_theses/49.
[196] K. Le Blanc, F. Frassoni, L. Ball, F. Locatelli, H. Roelofs, I. Lewis, E. Lanino, B. Sundberg, M.E. Bernardo, M. Remberger, G. Dini, R.M. Egeler, A. Bacigalupo, W. Fibbe, O. Ringdén, Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study, Lancet 371 (2008) 1579–1586, https://doi.org/10.1016/S0140-6736(08)60690-X.
[197] R.K. Burt, Y. Loh, W. Pearce, N. Beohar, W.G. Barr, R. Craig, Y. Wen, J.A. Rapp, J. Kessler, Clinical applications of blood-derived and marrow-derived stem cells for nonmalignant diseases, J. Am. Med. Assoc. 299 (2008) 925–936, https://doi.org/10.1001/jama.299.8.925.
[198] C. Toma, W.R. Wagner, S. Bowry, A. Schwartz, F. Villanueva, Fate of culture-expanded mesenchymal stem cells in the microvasculature: in vivo observations of cell kinetics, Circ. Res. 104 (2009) 398–402, https://doi.org/10.1161/CIRCRESAHA.108.187724.
[199] U.M. Fischer, M.T. Harting, F. Jimenez, W.O. Monzon-Posadas, H. Xue, S.I. Savitz, G.A. Laine, C.S. Cox Jr., Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect, Stem Cell. Dev. 18 (2009) 683–692, https://doi.org/10.1089/scd.2008.0253.
[200] M. Janowski, A. Lyczek, C. Engels, J. Xu, B. Lukomska, J.W. Bulte, P. Walczak, Cell size and velocity of injection are major determinants of the safety of intracarotid stem cell transplantation, J. Cerebr. Blood Flow Metabol. 33 (2013) 921–927, https://doi.org/10.1038/jcbfm.2013.32.
[201] J.M. Hare, J.H. Traverse, T.D. Henry, N. Dib, R.K. Strumpf, S.P. Schulman, G. Gerstenblith, A.N. DeMaria, A.E. Denktas, R.S. Gammon, J.B. Hermiller Jr., M.A. Reisman, G.L. Schaer, W. Sherman, A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction, J. Am. Coll. Cardiol. 54 (2009) 2277–2286, https://doi.org/10.1016/j.jacc.2009.06.055.
[202] P.R. Vulliet, M. Greeley, S.M. Halloran, K.A. MacDonald, M.D. Kittleson, Intracoronary arterial injection of mesenchymal stromal cells and microinfarction in dogs, Lancet 363 (2004) 783–784, https://doi.org/10.1016/S0140-6736(04)15695-X.
[203] M. Breitbach, T. Bostani, W. Roell, Y. Xia, O. Dewald, J.M. Nygren, J.W. Fries, K. Tiemann, H. Bohlen, J. Hescheler, A. Welz, W. Bloch, S.E. Jacobsen, B.K. Fleischmann, Potential risks of bone marrow cell transplantation into infarcted hearts, Blood 110 (2007) 1362–1369, https://doi.org/10.1182/blood-2006-12-063412.
[204] D. Cyranoski, Korean deaths spark inquiry, Nature 468 (2010) 485, https://doi.org/10.1038/468485a.
[205] L. Liao, B. Shi, H. Chang, X. Su, L. Zhang, C. Bi, Y. Shuai, X. Du, Z. Deng, Y. Jin, Heparin improves BMSC cell therapy: anticoagulant treatment by heparin improves the safety and therapeutic effect of bone marrow-derived mesenchymal stem cell cytotherapy, Theranostics 7 (2017) 106–116, https://doi.org/10.7150/thno.16911.
[206] B.M. Gleeson, K. Martin, M.T. Ali, A.H. Kumar, M.G. Pillai, S.P. Kumar, J.F. O'Sullivan, D. Whelan, A. Stocca, W. Khider, F.P. Barry, T. O'Brien, N.M. Caplice, Bone marrow-derived mesenchymal stem cells have innate procoagulant activity and cause microvascular obstruction following intracoronary delivery: amelioration by antithrombin therapy, Stem Cell. 33 (2015) 2726–2737, https://doi.org/10.1002/stem.2050.
[207] N. Baker, L.B. Boyette, R.S. Tuan, Characterization of bone marrow-derived mesenchymal stem cells in aging, Bone 70 (2015) 37–47, https://doi.org/10.1016/j.bone.2014.10.014.
[208] J. Kim, J.W. Kang, J.H. Park, Y. Choi, K.S. Choi, K.D. Park, D.H. Baek, S.K. Seong, H.K. Min, H.S. Kim, Biological characterization of long-term cultured human mesenchymal stem cells, Arch Pharm. Res. (Seoul) 32 (2009) 117–126, https://doi.org/10.1007/s12272-009-1125-1 117–126, 2009; 32.
[209] L. Zhou, X. Chen, T. Liu, Y. Gong, S. Chen, G. Pan, W. Cui, Z.P. Luo, M. Pei, H. Yang, F. He, Melatonin reverses H2O2-induced premature senescence in mesenchymal stem cells via the SIRT1-dependent pathway, J. Pineal Res. 59 (2015) 190–205, https://doi.org/10.1111/jpi.12250.
[210] H.M. Zhang, Y. Zhang, Melatonin: a well-documented antioxidant with conditional pro-oxidant actions, J. Pineal Res. 57 (2014) 131–146, https://doi.org/10.1111/jpi.12162.
[211] F. Luchetti, B. Canonico, M. Betti, M. Arcangeletti, F. Pilolli, M. Piroddi, L. Canesi, S. Papa, F. Galli, Melatonin signaling and cell protection function, Faseb. J. 24 (2010) 3603–3624, https://doi.org/10.1096/fj.10-154450.
[212] H. Koyama, O. Nakade, Y. Takada, T. Kaku, K.H. Lau, Melatonin at pharmacologic doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediated osteoclast formation and activation, J. Bone Miner. Res. 17 (2002) 1219–1229, https://doi.org/10.1359/jbmr.2002.17.7.1219.
[213] J.H. Son, Y.C. Cho, I.Y. Sung, I.R. Kim, B.S. Park, Y.D. Kim, Melatonin promotes osteoblast differentiation and mineralization of MC3T3-E1 cells under hypoxic conditions through activation of PKD/p38 pathways, J. Pineal Res. 57 (2014) 385–392, https://doi.org/10.1111/jpi.12177.
[214] D.P. Cardinali, A.I. Esquifino, V. Srinivasan, S.R. Pandi-Perumal, Melatonin and the immune system in aging, Neuroimmunomodulation 15 (2008) 272–278, https://doi.org/10.1159/000156470.
[215] J. Espino, J.A. Pariente, A.B. Rodríguez, Oxidative stress and immunosenescence: therapeutic effects of melatonin, Oxid. Med. Cell. Longev. (2012) 670294, https://doi.org/10.1155/2012/670294 2012.
[216] P.M. Mountziaris, P.P. Spicer, F.K. Kasper, A.G. Mikos, Harnessing and modulating inflammation in strategies for bone regeneration, Tissue, Eng. Part. B. Rev. 17 (2011) 393–402, https://doi.org/10.1089/ten.TEB.2011.0182.
[217] Y. Liu, C. Chen, S. Liu, D. Liu, X. Xu, X. Chen, S. Shi, Acetylsalicylic acid treatment improves differentiation and immunomodulation of SHED, J. Dent. Res. 94 (2015) 209–218, https://doi.org/10.1177/0022034514557672.
[218] J.M. Kelm, M. Fussenegger, Scaffold-free cell delivery for use in regenerative medicine, Adv. Drug Deliv. Rev. 62 (2010) 753–764, https://doi.org/10.1016/j.addr.2010.02.003.
[219] M. Ishijima, M. Hirota, W. Park, M.J. Honda, N. Tsukimura, K. Isokawa, T. Ishigami, T. Ogawa, Osteogenic cell sheets reinforced with photofunctionalized micro-thin titanium, J. Biomater. Appl. 29 (2015) 1372–1384, https://doi.org/10.1177/0885328214567693.
[220] M. Crisan, S. Yap, L. Casteilla, C.W. Chen, M. Corselli, T.S. Park, G. Andriolo, B. Sun, B. Zheng, L. Zhang, C. Norotte, P.N. Teng, J. Traas, R. Schugar, B.M. Deasy, S. Badylak, H.J. Buhring, J.P. Giacobino, L. Lazzari, J. Huard, B. Péault, A perivascular origin for mesenchymal stem cells in multiple human organs, Cell. Stem. Cell. 3 (2008) 301–313, https://doi.org/10.1016/j.stem.2008.07.003.
[221] S. Méndez-Ferrer, T.V. Michurina, F. Ferraro, A.R. Mazloom, B.D. Macarthur, S.A. Lira, D.T. Scadden, A. Ma'ayan, G.N. Enikolopov, P.S. Frenette, Mesenchymal and haematopoietic stem cells form a unique bone marrow niche, Nature 466 (2010) 829–834, https://doi.org/10.1038/nature09262.
[222] H. Zhao, J. Feng, K. Seidel, S. Shi, O. Klein, P. Sharpe, Y. Chai, Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor, Cell. Stem. Cell. 14 (2014) 160–173, https://doi.org/10.1016/j.stem.2013.12.013.
[223] S.C. Mendes, M. Sleijster, A. Van Den Muysenberg, J.D. De Bruijn, C.A. Van Blitterswijk, A cultured living bone equivalent enhances bone formation when compared to a cell seeding approach, J. Mater. Sci. Mater. Med. 13 (2002) 575–581, https://doi.org/10.1023/a:1015178827323.

[224] H.L. Holtorf, J.A. Jansen, A.G. Mikos, Ectopic bone formation in rat marrow stromal cell/titanium fiber mesh scaffold constructs: effect of initial cell phenotype, Biomaterials 26 (2005) 6208–6216, https://doi.org/10.1016/j.biomaterials.2005.04.006.
[225] M. Yamato, T. Okano, Cell sheet engineering, Mater. Today 7 (2004) 42.
[226] K. Ohashi, T. Yokoyama, M. Yamato, H. Kuge, H. Kanehiro, M. Tsutsumi, T. Amanuma, H. Iwata, J. Yang, T. Okano, Y. Nakajima, Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets, Nat. Med. 13 (2007) 880–885, https://doi.org/10.1038/nm1576.
[227] Y. An, W. Wei, H. Jing, L. Ming, S. Liu, Y. Jin, Bone marrow mesenchymal stem cell aggregate: an optimal cell therapy for full-layer cutaneous wound vascularization and regeneration, Sci. Rep. 5 (2015) 17036, https://doi.org/10.1038/srep17036.
[228] Z.H. Yang, F. Jin, X.J. Zhang, X. Liu, Y.F. Zhang, J.Q. Liu, Y.Z. Duan, Y. Jin, A novel possible strategy based on self-assembly approach to achieve complete periodontal regeneration, Artif. Organs 34 (2010) 603–609, https://doi.org/10.1111/j.1525-1594.2009.00991.x.
[229] J. Yang, M. Yamato, K. Nishida, T. Ohki, M. Kanzaki, H. Sekine, T. Shimizu, T. Okano, Cell delivery in regenerative medicine: the cell sheet engineering approach, J. Contr. Release 116 (2006) 193–203, https://doi.org/10.1016/j.jconrel.2006.06.022.
[230] I. Ishikawa, T. Iwata, K. Washio, T. Okano, T. Nagasawa, K. Iwasaki, T. Ando, Cell sheet engineering and other novel cell-based approaches to periodontal regeneration, Periodontol 51 (2000) 220–238, https://doi.org/10.1111/j.1600-0757.2009.00312.x 2009.
[231] F. Wei, C. Qu, T. Song, G. Ding, Z. Fan, D. Liu, Y. Liu, C. Zhang, S. Shi, S. Wang, Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity, J. Cell. Physiol. 227 (2012) 3216–3224, https://doi.org/10.1002/jcp.24012.
[232] C.T. Brighton, A.G. Krebs, Oxygen tension of nonunion of fractured femurs in the rabbit, Surg. Gynecol. Obstet. 135 (1972) 379–385.
[233] J. Dai, A.B. Rabie, VEGF: an essential mediator of both angiogenesis and endochondral ossification, J. Dent. Res. 86 (2007) 937–950, https://doi.org/10.1177/154405910708601006.
[234] M. Akahane, A. Nakamura, H. Ohgushi, H. Shigematsu, Y. Dohi, Y. Takakura, Osteogenic matrix sheet-cell transplantation using osteoblastic cell sheet resulted in bone formation without scaffold at an ectopic site, J. Tissue. Eng. Regen. Med. 2 (2008) 196–201, https://doi.org/10.1002/term.81.
[235] H.L. Wong, W.S. Siu, W.T. Shum, S. Gao, P.C. Leung, C.H. Ko, Application of Chinese herbal medicines to revitalize adult stem cells for tissue regeneration, Chin, J. Integr. Med. 18 (2012) 903–908, https://doi.org/10.1007/s11655-012-1293-3.
[236] J.F. Zhang, G. Li, C.Y. Chan, C.L. Meng, M.C. Lin, Y.C. Chen, M.L. He, P.C. Leung, H.F. Kung, Flavonoids of Herba Epimedii regulate osteogenesis of human mesenchymal stem cells through BMP and Wnt/beta-catenin signaling pathway, Mol. Cell. Endocrinol. 314 (2010) 70–74, https://doi.org/10.1016/j.mce.2009.08.012.
[237] P. Zhuang, Y. Zhang, G. Cui, Y. Bian, M. Zhang, J. Zhang, Y. Liu, X. Yang, A.O. Isaiah, Y. Lin, Y. Jiang, Direct stimulation of adult neural stem/progenitor cells in vitro and neurogenesis in vivo by salvianolic acid B, PloS One 7 (2012) e35636, , https://doi.org/10.1371/journal.pone.0035636.
[238] P. Zhang, K.R. Dai, S.G. Yan, W.Q. Yan, C. Zhang, D.Q. Chen, B. Xu, Z.W. Xu, Effects of naringin on the proliferation and osteogenic differentiation of human bone mesenchymal stem cell, Eur. J. Pharmacol. 607 (2009) 1–5, https://doi.org/10.1016/j.ejphar.2009.01.035.
[239] J.L. Kim, S.H. Park, D. Jeong, J.S. Nam, Y.H. Kang, Osteogenic activity of silymarin through enhancement of alkaline phosphatase and osteocalcin in osteoblasts and tibia-fractured mice, Exp. Biol. Med. 237 (2012) 417–428, https://doi.org/10.1258/ebm.2011.011376.
[240] L.N. Gao, Y. An, M. Lei, B. Li, H. Yang, H. Lu, F.M. Chen, Y. Jin, The effect of the coumarin-like derivative osthole on the osteogenic properties of human periodontal ligament and jaw bone marrow mesenchymal stem cell sheets, Biomaterials 34 (2013) 9937–9951, https://doi.org/10.1016/j.biomaterials.2013.09.017.
[241] D.Z. Tang, W. Hou, Q. Zhou, M. Zhang, J. Holz, T.J. Sheu, T.F. Li, S.D. Cheng, Q. Shi, S.E. Harris, D. Chen, Y.J. Wang, Osthole stimulates osteoblast differentiation and bone formation by activation of beta-catenin-BMP signaling, J. Bone Miner. Res. 25 (2010) 1234–1245, https://doi.org/10.1002/jbmr.21.
[242] H.S. Wang, S.C. Hung, S.T. Peng, C.C. Huang, H.M. Wei, Y.J. Guo, Y.S. Fu, M.C. Lai, C.C. Chen, Mesenchymal stem cells in the wharton's jelly of the human umbilical cord, Stem Cell. 22 (2004) 1330–1337, https://doi.org/10.1634/stemcells.2004-0013.
[243] F. Shang, S. Liu, L. Ming, R. Tian, F. Jin, Y. Ding, Y. Zhang, H. Zhang, Z. Deng, Y. Jin, Human umbilical cord MSCs as new cell sources for promoting periodontal regeneration in inflammatory periodontal defect, Theranostics 7 (2017) 4370–4382, https://doi.org/10.7150/thno.19888.
[244] A. Fleury, M.C. Martinez, S. Le Lay, Extracellular vesicles as therapeutic tools in cardiovascular diseases, Front. Immunol. 5 (2014) 370, https://doi.org/10.3389/fimmu.2014.00370.
[245] M. Mittelbrunn, F. Sánchez-Madrid, Intercellular communication: diverse structures for exchange of genetic information, Nat. Rev. Mol. Cell Biol. 13 (2012) 328–335, https://doi.org/10.1038/nrm3335.
[246] G. Ren, L. Zhang, X. Zhao, G. Xu, Y. Zhang, A.I. Roberts, R.C. Zhao, Y. Shi, Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide, Cell. Stem. Cell. 2 (2008) 141–150, https://doi.org/10.1016/j.stem.2007.11.014.
[247] T. Furuta, S. Miyaki, H. Ishitobi, T. Ogura, Y. Kato, N. Kamei, K. Miyado, Y. Higashi, M. Ochi, Mesenchymal stem cell-derived exosomes promote fracture healing in a mouse model, Stem. Cells. Transl. Med. 5 (2016) 1620–1630, https://doi.org/10.5966/sctm.2015-0285.
[248] J. Zhang, X. Liu, H. Li, C. Chen, B. Hu, X. Niu, Q. Li, B. Zhao, Z. Xie, Y. Wang, Exosomes/tricalcium phosphate combination scaffolds can enhance bone regeneration by activating the PI3K/Akt signaling pathway, Stem Cell Res. Ther. 7 (2016) 136, https://doi.org/10.1186/s13287-016-0391-3.
[249] X. Qi, J. Zhang, H. Yuan, Z. Xu, Q. Li, X. Niu, B. Hu, Y. Wang, X. Li, Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells repair critical-sized bone defects through enhanced angiogenesis and osteogenesis in osteoporotic rats, Int. J. Biol. Sci. 12 (2016) 836–849, https://doi.org/10.7150/ijbs.14809.
[250] S.H. Ranganath, O. Levy, M.S. Inamdar, J.M. Karp, Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease, Cell. Stem. Cell. 10 (2012) 244–258, https://doi.org/10.1016/j.stem.2012.02.005.
[251] S. Rani, A.E. Ryan, M.D. Griffin, T. Ritter, Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications, Mol. Ther. 23 (2015) 812–823, https://doi.org/10.1038/mt.2015.44.
[252] M. Osugi, W. Katagiri, R. Yoshimi, T. Inukai, H. Hibi, M. Ueda, Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects, Tissue Eng. 18 (2012) 1479–1489, https://doi.org/10.1089/ten.TEA.2011.0325.
[253] X. Liu, Q. Li, X. Niu, B. Hu, S. Chen, W. Song, J. Ding, C. Zhang, Y. Wang, Exosomes secreted from human-induced pluripotent stem cell-derived mesenchymal stem cells prevent osteonecrosis of the femoral head by promoting angiogenesis, Int. J. Biol. Sci. 13 (2017) 232–244, https://doi.org/10.7150/ijbs.16951.
[254] J.H. An, H. Park, J.A. Song, K.H. Ki, J.Y. Yang, H.J. Choi, S.W. Cho, S.W. Kim, S.Y. Kim, J.J. Yoo, W.Y. Baek, J.E. Kim, S.J. Choi, W. Oh, C.S. Shin, Transplantation of human umbilical cord blood-derived mesenchymal stem cells or their conditioned medium prevents bone loss in ovariectomized nude mice, Tissue, Eng. Part. A. 19 (2013) 685–696, https://doi.org/10.1089/ten.TEA.2012.0047.
[255] C.Y. Wang, H.B. Yang, H.S. Hsu, L.L. Chen, C.C. Tsai, K.S. Tsai, T.L. Yew, Y.H. Kao, S.C. Hung, Mesenchymal stem cell-conditioned medium facilitates angiogenesis and fracture healing in diabetic rats, J. Tissue. Eng. Regen. Med. 6 (2012) 559–569, https://doi.org/10.1002/term.461.
[256] H. Peinado, M. Alečković, S. Lavotshkin, I. Matei, B. Costa-Silva, G. Moreno-Bueno, M. Hergueta-Redondo, C. Williams, G. García-Santos, C. Ghajar, A. Nitadori-Hoshino, C. Hoffman, K. Badal, B.A. Garcia, M.K. Callahan, J. Yuan, V.R. Martins, J. Skog, R.N. Kaplan, M.S. Brady, D. Lyden, Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET, Nat. Med. 18 (2012) 883–891, https://doi.org/10.1038/nm.2753.
[257] R.J. Lobb, M. Becker, S.W. Wen, C.S. Wong, A.P. Wiegmans, A. Leimgruber, A. Möller, Optimized exosome isolation protocol for cell culture supernatant and human plasma, J. Extracell. Vesicles 4 (2015) 27031, https://doi.org/10.3402/jev.v4.27031.
[258] Y. Qin, R. Sun, C. Wu, L. Wang, C. Zhang, Exosome: a novel approach to stimulate bone regeneration through regulation of osteogenesis and angiogenesis, Int. J. Mol. Sci. 17 (2016) 712, https://doi.org/10.3390/ijms17050712.
[259] G. Dou, R. Tian, X. Liu, P. Yuan, Q. Ye, J. Liu, S. Liu, J. Zhou, Z. Deng, X. Chen, S. Liu, Y. Jin, Chimeric apoptotic bodies functionalized with natural membrane and modular delivery system for inflammation modulation, Sci. Adv. 6 (2020) eaba2987.
[260] Y. Li, L. Fan, S. Liu, W. Liu, H. Zhang, T. Zhou, D. Wu, P. Yang, L. Shen, J. Chen, Y. Jin, The promotion of bone regeneration through positive regulation of angiogenic-osteogenic coupling using microRNA-26a, Biomaterials 34 (2013) 5048–5058, https://doi.org/10.1016/j.biomaterials.2013.03.052.
[261] Y. Li, L. Fan, J. Hu, L. Zhang, L. Liao, S. Liu, D. Wu, P. Yang, L. Shen, J. Chen, Y. Jin, MiR-26a rescues bone regeneration deficiency of mesenchymal stem cells derived from osteoporotic mice, Mol. Ther. 23 (2015) 1349–1357, https://doi.org/10.1038/mt.2015.101.
[262] B.D. Sui, C.H. Hu, A.Q. Liu, C.X. Zheng, K. Xuan, Y. Jin, Stem cell-based bone regeneration in diseased microenvironments: challenges and solutions, Biomaterials 196 (2017) 18–30, https://doi.org/10.1016/j.biomaterials.2017.10.046.