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Review article

THE ROLE OF BONE MARROW CELLS AND PERIPHERAL BLOOD CELLS IN THE OSTEOGENIC PROCESS

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The osteogenic process is a complex and dynamic biological phenomenon essential for the initial formation of bones during embryonic development and the continuous remodeling and repair of bones throughout an individual's life. It involves coordination of various cell types, signaling pathways, and environmental factors to ensure proper bone formation and maintenance. The main role in this process belongs to bone marrow cells and peripheral blood cells. This paper provides an overview of currently available literature data about different contributions of bone marrow cells and peripheral blood cells to the osteogenic process. Focusing on their differentiation, signaling pathways, and interactions within the bone microenvironment this article aims to provide a comprehensive understanding of how these cells orchestrate the osteogenic process, offering insights into their therapeutic potential. Understanding these complex cellular interactions is crucial for the development of advanced therapeutic approaches in regenerative medicine and orthopedics, which will ultimately improve outcomes in patients with bone defects and bone-related disorders.

Keywords: bone marrow cells, peripheral blood cells, osteogenic process

INTRODUCTION

The bones represent basic components of the skeletal system in all vertebrates which serves as a support for the rest of the body, allows body movements and protection of vital organs and whose integrity is often damaged by bone defects [1]. Bone defects are commonly consequence of trauma, tumors, congenital malformations or diseases [2]. Since their presence greatly disrupts the quality of life and very often leads to permanent disability of a patient, a significant medical challenge for orthopedic and maxillofacial surgery represents the reconstruction of bone defects. Transplantations of autologous bone grafts were indicated as the gold standard in the treatment of bone defects [2-4]. However, autotransplantation has disadvantages such as the

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necessity of another surgical intervention, small amount of available bone, risk of infection, and donor site morbidity. Alternatives to autotransplantation in many cases involve bone substitutes such as allogenic bone and xenogenic bone substitutes, and also the use of different biomaterials [2,3]. All of them possess limitations and risks in their application. These challenges have directed extensive researches, primarily conducted on animal models, towards the development of alternative strategies for bone regeneration and repair, among which cell-based therapies have a great promising potential [5]. The use of appropriate animal models (commonly rodents as small animal and dogs, sheep and pigs as large animals) is critical to improve current treatment methods and develop novel rescue therapies [6-8]. In order to translate the results obtained on animal models to humans, it is necessary to take into account the anatomical and histological differences as well as differences in physiological and biochemical processes [1,9].

It is known that beside bone marrow cells, peripheral blood cells are also involved in osteogenic processes and those different cells by different mechanisms contribute to bone formation [10-12]. Thus, understanding the intricate interplay between these cells, both *in vitro* and *in vivo*, is of paramount importance for developing cell-based therapeutic strategies for the reconstruction of bone defects [5].

The central theme of this work is the characterization of bone marrow and blood cells, their contribution and mutual interaction in bone formation, homeostasis and repair as a basis for further development of alternative strategies for bone regeneration. By integrating findings from basic and translational research, a comprehensive overview of cellular interactions underlying osteogenesis is provided, with the therapeutic potential of bone marrow and blood cells for the treatment of bone defects and bone-related disorders. Also, this review provides key insights into the fundamental processes of bone biology, contributing to a broader understanding of bone physiology and pathology.

BONE MARROW CELLS

Bone marrow represents a vital tissue located within the cavities of bones, primarily of the axial skeleton, including the pelvis, sternum, and vertebrae. The composition of bone marrow is very complex and consists of various cells and different extracellular matrix components which play critical roles both in hematopoiesis and osteogenesis [13-16]. According to the histological findings and function it is classified into two types: red bone marrow and yellow bone marrow. Red bone marrow is primarily involved in the production of blood cells, including erythrocytes, leukocytes, and platelets [17], while the yellow bone marrow, mainly includes adipocytes and serves as a reservoir for fats. Under specific conditions, such as severe blood loss or anemia the yellow bone marrow can transform back into red bone marrow [13].

Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) are adult multipotent stromal progenitor cells that reside in the bone marrow. They have a crucial role in the osteogenic process because they possess the capacity to differentiate into various cell types, including osteoblasts, chondrocytes, and adipocytes in response to specific signals and microenvironmental cues [13,15]. They contribute to tissue regeneration by secreting various growth factors and cytokines that recruit endogenous progenitor cells to the injury site and regulate the function of other cells, including osteoclasts and endothelial cells, thereby influencing bone remodelling and vascularization [18-20]. Their unique characteristics allows them to perform their physiological roles in the organism and also make them promising candidates for regenerative medicine and tissue engineering applications.

Characteristics of MSCs

In the body, the MSC population is maintained by continuous self-renewal throughout an individual's lifetime as a life-long source of progenitor cells. Some of these cells undergo continuous cell division where one part of them retains their undifferentiated state and ability to divide and the other part undergoes differentiation in response to specific environmental factors [13,15,18]. Apart from their direct role in restoring the fund of tissue and organ cells, MSC also exhibit immunomodulatory properties, allowing them to interact with immune cells and modulate the inflammatory response [10].

Since, MSCs have the ability to differentiate into different cells of mesodermal lineage as well as ectodermal (neurocytes) and endodermal lineages (hepatocytes) [10,15,18], this multilineage *in vitro* and *in vivo* differentiation potential makes them crucial in the maintenance and remodelling of different tissues, especially in bone remodelling and regeneration [21].

One of the primary morphological features of MSCs is their elongated, spindle-shaped, fibroblast-like appearance when observed under a microscope which is consistent across various sources of MSCs [15,18,21]. Given that these cells do not have a unique morphology and it is not possible to distinguish them according only to their morphology, thus there were several attempts to characterize MSCs according to the expression of cell surface markers. Finally, it is accepted that characteristics of MSCs is the expression of CD73, CD90, and CD105, while they lack the expression of CD14, CD34, CD45 and HLA (human leukocyte antigen)-DR [21].

Sources of MSCs

Primarily, MSCs were identified in the bone marrow, where they reside in a specialized microenvironment called the "stem cell niche" [13,18]. Subsequent studies have demonstrated that MSCs can also be isolated from various other tissues. Adipose-derived stem cells (ASCs) are stem cells isolated from adipose tissue that share many

similarities with bone marrow-derived MSCs (BM-MSCs) in terms of differentiation potential, immunomodulatory properties and regenerative capacity [22]. Unlike BM-MSCs, ASCs can be obtained from abundant adipose tissue by a minimally invasive procedure which gives high yield of cells. Different works reported that ASCs possess not only osteoconductive capacity, but also osteoinductive capacity that recruits adipose tissue as a potential and rich source of MSCs for further clinical application [22,23]. MSCs are also isolated from different other tissues such as the umbilical cord, different dental tissues, placenta, extraocular muscle, and ocular adipose tissue. It is shown that the highest osteogenic differentiation potential hold the bone marrow MSCs, while the differentiation potential and potential application of MSCs cells isolated from other tissues depends on the tissue source they are isolated from [18,21].

From mesenchymal stem cells to osteoblasts

The osteogenic differentiation of MSCs into osteoblasts represents a complex series of events influenced by various factors within their microenvironment, including cell-cell interactions, paracrine factors, mechanical stimuli, hormones, and cytokines [18]. These factors activate numerous signaling molecules associated with different molecular pathways such as bone morphogenetic proteins (BMPs), NOTCH, WNT, HEDGEHOG, and NELL-1 [24]. These pathways, both independently and in conjunction with other signaling molecules, regulate the differentiation of MSCs into osteoblasts by triggering specific osteogenic transcription factors that ultimately lead to the expression of osteoblast-specific genes or play an inhibitory role such as BMP13 and NOTCH [24].

As a key transcription factor, MSCs begin to express core-binding factor alpha-1 (CBFA1, also known as RUNX2), which marks the commitment of MSCs to the osteoblast lineage. RUNX2 drives the expression of osteoblast-specific genes, such as genes for alkaline phosphatase (ALP), osteocalcin (OCN), and bone sialoprotein (BSP), which promote the deposition and mineralization of the bone matrix [25]. As these osteoblasts mature and continue to secrete the bone matrix, some become embedded within the matrix and differentiate further into osteocytes. This transition is marked by the downregulation of osteoblast markers like ALP and OCN, and the upregulation of osteocyte-specific markers such as sclerostin (SOST) and dentin matrix protein 1 (DMP1) (Figure 1) [25].

For a potential therapeutic application, MSCs can be used immediately after isolation or they can be pre-conditioned or combined with other cells and signaling molecules in order to activate osteo-promoting signaling and to enhance the therapeutic benefits before cell transplantation [21]. Current researches predominantly focus on using MSCs for bone regeneration as local MSC injection or application in combination with scaffolds. Despite the progress, local application has limitations particularly for systemic conditions like multiple fractures and osteoporosis. In such cases, systemic administration of MSCs is considered more promising, practical and suitable, but still has a lot of challenges to be overcome [26].

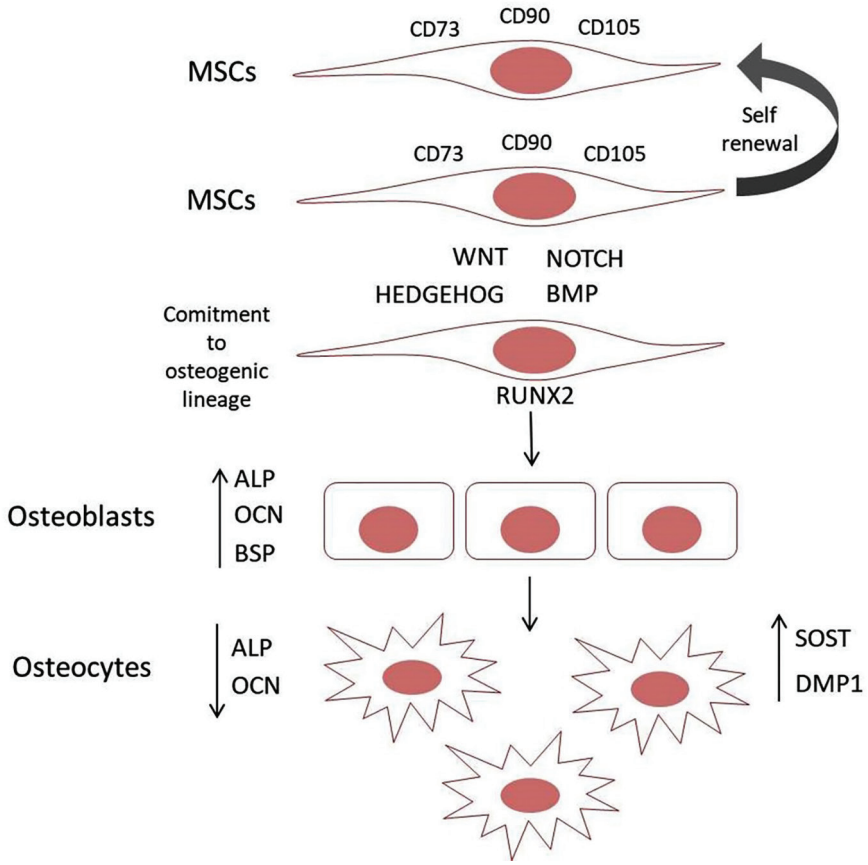

Figure 1. Characteristics of MSCs and their differentiation toward osteogenic lineage. **ALP:** alkaline phosphatase; **OCN:** osteocalcin; **BSP:** bone sialoprotein; **SOST:** sclerostin; **DMP1:** dentin matrix protein 1

Hematopoietic Stem Cells (HSCs)

Hematopoietic stem cells (HSCs) represent rare multipotent stem cells that reside in the bone marrow and which are primarily responsible for the continuous production of blood and immune cells throughout an individual's life [27]. Beside their primary role in hematopoiesis, they possess the capability to differentiate into osteoclasts, that is cells that are vital for the osteogenic process [28,29].

Characteristics of HSCs

One of the main characteristics of HSCs is their multipotency [27,30]. HSCs undergo a series of divisions and differentiations to produce multipotent progenitor cells (MPPs), which retain the ability to differentiate into multiple lineages but have limited self-renewal capacity compared to HSCs. MPPs further differentiate into lineage-restricted progenitors, such as common myeloid progenitors (CMPs) and common

lymphoid progenitors (CLPs). CMPs give rise to myeloid cells, including granulocytes, monocytes, erythrocytes, and megakaryocytes, while CLPs differentiate into lymphoid cells, such as T cells, B cells, and natural killer (NK) cells [31].

Within the population of HSCs, there are two distinct subsets: long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs). These subsets are defined based on their capacity for self-renewal and their contribution to hematopoiesis over time. LT-HSCs are characterized by their extensive self-renewal capacity and ability to sustain hematopoiesis throughout an individual's lifetime. These cells reside predominantly in the bone marrow niche and are typically quiescent, dividing infrequently to maintain the stem cell pool and minimize the risk of accumulating genetic damage [32,33]. The quiescent state of LT-HSCs is regulated by various intrinsic factors, such as transcription factors and epigenetic regulators, as well as extrinsic signals from the bone marrow microenvironment [27,33]. On the contrary, ST-HSCs have a limited self-renewal capacity and are primarily responsible for the rapid replenishment of blood cells following injury or stress. These cells are more proliferative than LT-HSCs, giving rise to MPPs that further differentiate into all blood cell lineages. ST-HSCs are crucial in maintaining hematopoiesis over shorter periods, typically spanning several weeks to months [17].

The bone marrow niche provides essential extrinsic signals that regulate HSC behavior. These signals include cytokines, growth factors, and interactions with niche cells such as osteoblasts and MSCs [33]. Osteoblasts regulate HSC quiescence and proliferation through the secretion of factors such as osteopontin (OPN) and angiopoietin-1 (ANG-1), while endothelial cells contribute to the vascular niche, providing signals that support HSC maintenance and mobilization [13,17] (Figure 2).

The NOTCH signalling pathway plays a significant role in maintaining HSC quiescence and preventing premature differentiation, while the WNT signalling pathway is involved in both HSC self-renewal and differentiation. CXCL12, or stromal cell-derived factor 1 (SDF-1), is produced by stromal cells and osteoblasts in the bone marrow. It binds to the CXCR4 receptor on HSCs, facilitating their retention in the niche and regulating their localization and migration [17].

Under a microscope, HSCs appear similar to lymphocytes, with a slightly irregular, condensed chromatin pattern, and often display a lack of prominent nucleoli. Due to their morphological similarity to other hematopoietic cells, HSCs are primarily identified by a combination of surface markers and functional characteristics rather than morphology alone. It is accepted that in humans HSCs can be identified and isolated based on the expression of specific positive cell surface markers as CD34, CD49f, CD90 and EPCR [34] (Figure 2).

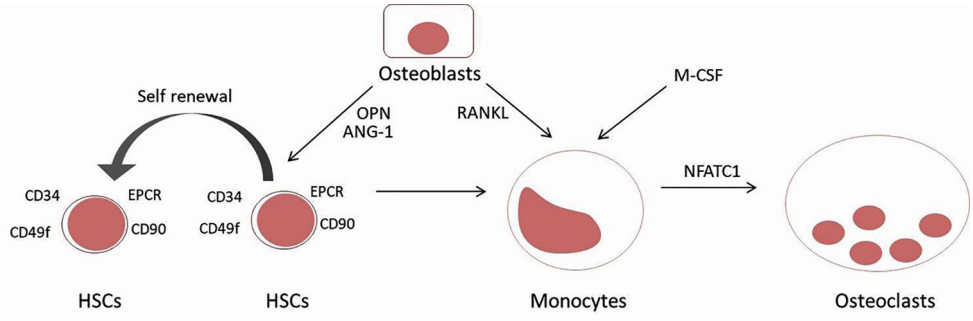

Figure 2. Characteristics of HSCs and their differentiation toward osteoclasts. **OPN:** osteopontin; **ANG-1:** angiopoietin-1; **RANKL:** receptor activator of nuclear factor kappa-B ligand; **M-CSF:** macrophage colony-stimulating factor; **NFATc1:** nuclear factor of activated T-cells, cytoplasmic 1

Sources of HSCs

The bone marrow is the primary site of HSC residence in adults, where they reside within specialized microenvironments known as the stem cell niche [17]. Beside the bone marrow, HSCs are isolated from newborns' umbilical cord blood, which has gained significant attention due to its ease of collection, lower risk of graft-versus-host disease, and higher proliferative capacity compared to adult bone marrow-derived HSCs [35,36]. Also, HSCs can be mobilized from the bone marrow into the peripheral blood following treatment with specific growth factors, such as granulocyte-colony stimulating factor (G-CSF). These mobilized HSCs can then be collected via apheresis and used for transplantation [37].

From hematopoietic stem cells to osteoclasts

The differentiation of HSCs into osteoclasts is essential in bone remodeling and requires a complex interplay of signaling pathways and molecular regulators.

Osteoclastogenesis begins with the differentiation of HSCs into monocytes, a process regulated by several cytokines [17]. Once monocytes are formed, they can differentiate into osteoclasts under the influence of two critical cytokines: macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) [29,38,39]. M-CSF binds to its receptor on monocytes, leading to their proliferation and survival. RANKL, produced by osteoblasts and bone marrow stromal cells, binds to its receptor RANK on the surface of monocytes, triggering a signaling cascade that results in the activation of several transcription factors, including nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1). NFATc1 is considered the master regulator of osteoclastogenesis, as it induces the expression of various osteoclast-specific genes, leading to the formation of mature osteoclasts [40] (Figure 2).

Osteoclastogenesis is also regulated by other signaling molecules such as osteoprotegerin (OPG), a decoy receptor for RANKL which inhibits its interaction with RANK,

thereby preventing osteoclastogenesis [40]. Thus, the balance between RANKL and OPG is crucial for regulating osteoclastogenesis and bone remodeling.

Additionally, the immune system plays an important role in osteoclastogenesis. Several immune-related signaling pathways, including those mediated by the tumor necrosis factor (TNF) family of cytokines, contribute to osteoclast formation and function [41,42]. It is also shown that macrophages isolated from different tissues in combination with different cells and materials can stimulate the osteogenic process [43,44].

Megakaryocytes

Megakaryocytes, the large bone marrow cells responsible for the production of platelets, have traditionally been associated with hemostasis. However, there are emerging evidence about their significant roles in the orchestration of bone formation and remodeling [45].

Characteristics and sources of megakaryocytes

Megakaryocytes are large, rounded or elongated cells with a multi-lobed nucleus, derived from the pluripotent hematopoietic stem cells (HSCs) residing in the bone marrow. Through the process of commitment and differentiation, these HSCs differentiate into megakaryocyte-erythroid progenitors (MEPs), which subsequently mature into megakaryocytes under the influence of the key growth factor, thrombopoietin (TPO) [46].

The role in osteogenesis

The cross-talk between megakaryocytes and osteoblasts contributes significantly to bone formation. Megakaryocytes express high levels of bone morphogenetic protein-6 (BMP-6) a potent inducer of osteoblast differentiation. BMP-6 binds to its receptors on osteoblasts, stimulating their differentiation and promoting bone formation. Studies have shown that megakaryocytes can stimulate also osteoblast proliferation and differentiation through the secretion of factors such as platelet-derived growth factor (PDGF) and fibroblast growth factor-2 (FGF-2). Megakaryocytes can also impact bone remodeling through interactions with osteoclasts by secretion of OPG which binds to RANKL. By binding RANKL, OPG inhibits osteoclast differentiation and function, thus reducing bone resorption [45].

Megakaryocytes are pivotal in the maintenance of the HSCs niche within the bone marrow influencing the location, proliferation, and differentiation of HSCs. Also, this niche regulatory role indirectly impacts osteogenesis, given the osteogenic potential of mesenchymal stem cells residing in the same bone marrow microenvironment [47] (Figure 3).

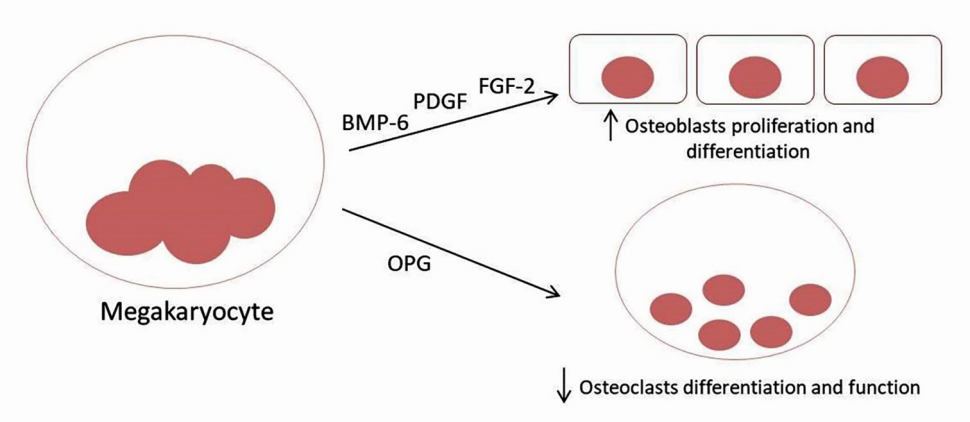

Figure 3. Characteristics of megakaryocytes and their influence on osteoblasts and osteoclasts. **BMP-6:** bone morphogenetic protein-6; **PDGF:** platelet-derived growth factor; **FGF-2:** fibroblast growth factor-2; **OPG:** osteoprotegerin

Endothelial progenitor cells (EPCs)

Endothelial progenitor cells (EPCs) represent a subset of stem cells derived from bone marrow and peripheral blood that can differentiate into endothelial cells. EPCs have recently gained attention for their role in osteogenesis due to their capacity to stimulate neovascularization and vascular repair, essential processes in bone repair and regeneration [48].

Sources and Characteristics of EPCs

One of the most probable ways of forming EPCs is that hemangioblasts arise from the mesoderm, which gives rise to angioblasts and hemogenic endothelium. Further, haemogenic endothelium gives rise to HSCs and EPCs [48].

EPCs can be found in almost all tissues in the organism. The bone marrow is the primary source of EPCs. Also, EPCs are present in the peripheral blood, although in much lower quantities than in the bone marrow. The concentration of circulating EPCs can increase in response to tissue injury or the administration of specific growth factors providing a readily accessible source of cells for therapeutic applications [49]. Umbilical cord blood is another source of EPCs, offering an abundant and non-invasive collection method. EPCs derived from umbilical cord blood have shown comparable functional abilities to those derived from bone marrow and peripheral blood [11,49].

One of the essential characteristics of EPCs is that they also have the ability to self-renewal, i.e., to divide to give also EPCs as well as endothelial cells. They don't have unique cell morphology but they are characterized by their expression of specific surface markers, such as CD31, CD34, CD133, CD146 and vascular endothelial growth factor receptor 2 (VEGFR2) which allow their identification and isolation

from various sources [50]. Additionally, EPCs have the unique capacity to take up acetylated low-density lipoprotein and to bind to *Ulex europaeus* agglutinin, further distinguishing them from other cell types [49].

The role in osteogenesis

Bone healing requires sufficient blood supply to deliver oxygen, nutrients, and progenitor cells to the injury site. EPCs migrate to the injury site and differentiate into endothelial cells, forming new blood vessels. This process of vasculogenesis is indispensable for effective bone repair [51]. The subtypes of endothelial cells, identified as H and L, contribute to the formation of specific bone microvessel subtypes, which play a crucial role in both bone formation and bone resorption [51,52]. During the initial stages of intramembranous and endochondral ossification, capillaries infiltrate the primary ossification site, delivering essential elements such as oxygen and influencing the process of osteogenesis [52].

Besides vasculogenesis, EPCs influence osteogenesis through their interactions with osteoblasts. Some studies have demonstrated that EPCs can secrete various pro-osteogenic growth factors such as BMPs that stimulate the proliferation and differentiation of osteoblasts [24,51,53] (Figure 4).

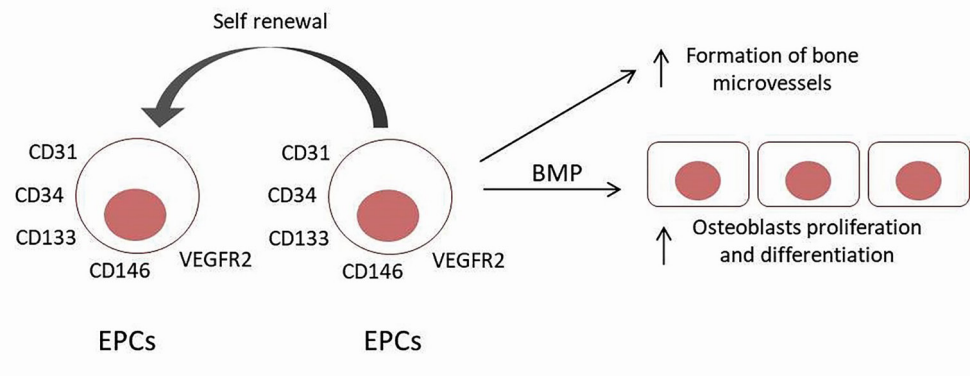

Figure 4. Characteristics of EPCs and their influence on bone repair. **VEGFR2:** vascular endothelial growth factor receptor 2; **BMP:** bone morphogenetic protein

Given their roles in vascularization and osteogenesis, EPCs have been explored as therapeutic targets for promoting bone repair. Mobilizing EPCs from the bone marrow or infusing *ex vivo* expanded EPCs has shown the potential to enhance bone healing in preclinical models [49,53].

PERIPHERAL BLOOD CELLS

Peripheral blood cells are a diverse group of cells circulating in the bloodstream, playing essential roles in various physiological processes, including oxygen transport, immune response, and blood clotting. Each type of cell has unique morphological characteristics and functions that are vital for maintaining homeostasis and protecting the body from diseases. Besides their primary roles, there is rising evidence about their role in bone remodelling and repair [54].

Peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) are a heterogeneous population of peripheral blood cells having round nuclei whose overall function ranges from immunity to inflammation. The lymphocytes within PBMCs play a significant role in immunity, while the monocytes and dendritic cells are crucial for inflammatory responses [11,55].

PBMCs potential role in osteogenesis has recently become a topic of interest [11] and current studies focus on their potential for differentiation into osteoblasts and osteoclasts and their interaction with other bone cells [55]. Since, PBMCs are found in peripheral blood they are easily accessible and isolating PBMCs offers a noninvasive method to collect a variety of cell types with potential roles in osteogenesis.

The role in osteogenesis

Circulating osteogenic precursor (COP) cells: Circulating osteogenic precursor (COP) cells represent a subset of blood-borne cells that possess osteogenic potential [56]. COP cells are found within the peripheral blood mononuclear cell (PBMC) fraction, constituting approximately 0.42% of this population. These cells circulate consistently throughout a healthy individual's life, with increased levels observed during periods of accelerated bone growth [11]. Research indicates that COP cells might originate from bone marrow MSCs mobilized into circulation in response to peripheral tissue demands. While the bone marrow is considered the most likely source of COP cells, their exact cellular lineage remains undetermined because these cells share similarities in behavior and appearance with bone MSCs, but they also uniquely express hematopoietic lineage markers. Despite this, the precise origin of COP cells is still debated [11]. According to one of the proposed models, both hematopoietic and mesenchymal lineages derive from a common ancestral progenitor cell, which could potentially be very small embryonic-like (VSEL) stem cells. These VSEL stem cells have shown the ability to regenerate tissue from all germ layers and might be candidates for this ancestral progenitor [57].

The study of Chen et al., 2023 resulted in *in vitro* reprogramming of human PBMCs into induced mesenchymal stromal cells [58]. While not traditionally considered a

source of osteogenic progenitor cells, these findings suggest that PBMCs can be used for obtaining of cells for further use in bone regeneration and repair.

Monocytes: It is previously mentioned that monocytes, a key subset of PBMCs for bone regeneration, are precursor cells to osteoclasts. The influence of key cytokines, such as M-CSF and RANKL, can drive the differentiation of monocytes into mature, bone-resorbing osteoclasts [41]. This process is crucial in bone remodelling and in pathological conditions characterized by excessive bone resorption.

Lymphocytes: Lymphocytes, particularly T cells, can secrete RANKL, promoting osteoclastogenesis. Conversely, B cells can also secrete OPG, a decoy receptor for RANKL, inhibiting osteoclastogenesis [59,60]. Thus, T and B cells can influence the balance between bone formation and resorption.

More research is needed to understand the full extent of PBMCs' role in osteogenesis. Potential of PBMCs to differentiate into osteoblast-like cells, coupled with its ability to regulate osteoclastogenesis, underscores their importance in bone homeostasis.

Platelets

Platelets are small, anucleate blood cells (cell fragments) derived from the cytoplasm of megakaryocytes. They have a primary role in hemostasis and also have been recognized for their significant contribution to bone healing and remodelling [61].

Characteristics of platelets

Platelets have a discoid shape, with a diameter ranging from 2-4 micrometers and contain multiple types of granules, namely alpha granules, dense granules, and lysosomes, filled with numerous bioactive molecules. They can adhere to sites of vascular injury and aggregate to form a hemostatic plug, a process essential for stopping bleeding [61].

Sources of platelets

Platelets are derived from megakaryocytes. Megakaryocytes originate from HSCs through a series of differentiation and maturation steps influenced by various cytokines, primarily TPO [46]. The production of platelets from megakaryocytes involves a series of complex and intriguing cellular processes. As megakaryocytes mature, they become polyploid leading to an increase in cell size and the accumulation of substantial amounts of protein and membrane. This phenomenon is followed by a cytoskeleton-driven mechanism where megakaryocytes extend long, branching processes called proplatelets into the sinusoidal blood vessels of the bone marrow. These proplatelets subsequently undergo fission, releasing mature platelets into the bloodstream [62].

The role in osteogenesis

Platelets have long been known for their critical role in hemostasis, but more recent studies have illuminated their vital participation in the healing and regeneration of tissues, including bone [63]. Their involvement in osteogenesis is primarily due to their ability to secrete a variety of growth factors and cytokines, such as PDGF, transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF). Upon platelet activation, these growth factors are released from the alpha granules, leading to the recruitment and proliferation of cells necessary for bone healing [64]. The growth factors stimulate the proliferation and migration of osteoblasts, endothelial cells, and MSCs to the site of bone injury. Furthermore, they have been shown to promote the differentiation of MSCs into osteoblasts, which action is fundamental for the repair and regeneration of bone tissue. The growth factors released by platelets, specifically VEGF, stimulate the formation of new blood vessels, providing the necessary nutrients and oxygen to the regenerating tissue [64].

In addition to bone healing and contribution to angiogenesis, platelets play a significant role in the field of bone grafting and biomaterial application. Platelet-rich plasma (PRP), a concentrated preparation of platelets, has been extensively used in combination with different cells and materials to improve graft or material incorporation and stimulate bone regeneration [65]. The growth factors contained in PRP enhance the osteogenic potential of the graft material, leading to improved bone healing and regeneration outcomes [66]. How strong is osteogenic potential of PRP show the work of Vukelić-Nikolić et al., where it is found that that not only PRP, but also diluted PRP possess osteoinductive potential and significantly boost the osteogenic process in ectopic bone forming model. This and similar findings recruit PRP as a potent inductor of osteogenic process in combination with different materials and cells which is of great importance for the further development of different therapeutic approaches in the regeneration of bone tissue [67]. As the normal number of platelets differs from species to species, in order for PRP to exhibit desired effects, it is necessary to determine the optimal protocol for the PRP preparation and optimal concentration of platelets for each species [68].

Erythrocytes

Erythrocytes, commonly known as red blood cells (RBCs), are biconcave discoid cells that primarily transport oxygen from the lungs to the tissues. However, recent researches indicate a potential function in bone formation and remodelling, expanding our understanding of erythrocytes beyond mere oxygen carriers [69,70].

Characteristics and sources of erythrocytes

Erythrocytes are generated from HSCs in the bone marrow through a process termed erythropoiesis [70]. The differentiation of HSCs into erythrocytes is a highly regulated process involving a cascade of lineage-specific progenitors, including the

megakaryocyte-erythroid progenitor and the erythroid progenitor. The glycoprotein hormone erythropoietin plays a vital role in erythropoiesis, regulating the erythroid progenitors' survival, proliferation, and differentiation [69].

The role in osteogenesis

While the specific mechanisms by which erythrocytes might influence bone homeostasis are yet to be fully elucidated, their role in oxygen transportation is crucial for the functioning of bone cells, particularly osteoblasts and osteoclasts. The metabolic activity of these cells is significantly affected by oxygen tension; hence, erythrocytes, through oxygen delivery, can indirectly influence the process of osteogenesis [71]. Hypoxia can lead to altered bone cell activity and, ultimately, bone loss, illustrating the importance of adequate oxygenation in maintaining healthy bones [69,71].

On the contrary to the indirect positive influence of undamaged erythrocytes on osteogenesis, there is also evidence of the negative impact of damaged erythrocytes on the osteogenic process. Namely, an *in vitro* study of Dregalla et al., 2021 showed that red blood cells and their releasates compromise bone marrow-derived human mesenchymal stem/stromal cell survival [72].

Recent studies indicate that mature erythrocytes can release extracellular vesicles containing microRNAs that can influence osteoblast and osteoclast function [73]. Neighbouring cells could take up erythrocyte-derived miRNAs and modulate their gene expression. In the context of bone remodeling, it is conceivable that erythrocyte-derived miRNAs might influence the activity of osteoblasts and osteoclasts. However, the specific miRNAs secreted by erythrocytes and their roles in osteogenesis are still unknown and represent an exciting area for future research.

CONCLUSION

Recent advances in stem cell biology and tissue engineering have opened new avenues for harnessing the regenerative potential of bone marrow and blood cells for bone repair and regeneration. This review has provided a comprehensive overview of the role of bone marrow and blood cells in the osteogenic process, highlighting the complex interplay between hematopoietic and mesenchymal cell populations in bone formation, homeostasis, and repair.

A deeper understanding of the role of bone marrow and blood cells in osteogenesis will advance our knowledge of bone tissue biology and make the basis for developing new therapeutic approaches for bone regeneration and repair. Emerging technologies such as cell-based therapies, biomaterials, and gene editing are promising to enhance bone regeneration strategies' efficacy and precision.

However, several challenges remain to be addressed, including optimizing cell sources, delivery methods, and tissue-engineering scaffolds, as well as overcoming

immunological barriers and ensuring long-term safety and efficacy. Future research efforts should focus on elucidating the dynamic interplay between bone marrow and blood cells in health and disease and translating these findings into innovative clinical applications.

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Author's contributions

MVN has made substantial contributions to the conception, design, and writing of the manuscript and, draw all figures, have been involved in revising the manuscript. LjĐ has contributed to the conception, design, and writing of the manuscript and has given final approval of the version to be published. All authors read and approved the final version.

Competing interest

The author(s) declare that they have no competing interests'.

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ULOGA ĆELIJA KOŠTANE SRŽI I ĆELIJA PERIFERNE KRVI U PROCESU OSTEOGENEZE

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Osteogeni proces je složen i dinamičan biološki fenomen koji leži u osnovi inicijalnog formiranja kostiju tokom embrionalnog razvoja kao i kontinuiranog remodelovanja i reparacije kostiju tokom života. On uključuje koordinaciju aktivnosti različitih tipova ćelija, signalnih puteva i faktora životne sredine kako bi se obezbedilo pravilno formiranje i održavanje kostiju tokom života. Glavna uloga u ovom procesu pripada ćelijama koštane srži i ćelijama periferne krvi. Ovaj rad daje pregled trenutno dostupnih literaturnih podataka o različitim doprinosima ćelija koštane srži i ćelija periferne

krvi osteogenom procesu. Fokusirajući se na diferencijaciju ćelija, signalne puteve i interakcije unutar mikrookruženja kosti, ovaj članak ima za cilj da pruži sveobuhvatno razumevanje kako ove ćelije orkestriraju osteogeni process istovremeno nudeći uvid u njihov terapijski potencijal. Razumevanje ovih složenih ćelijskih interakcija je ključno za razvoj naprednih terapijskih pristupa u regenerativnoj medicini i ortopediji, koje imaju za cilj poboljšanje ishoda kod pacijenata sa koštanim defektima i poremećajima vezanim za kosti.