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EXPERIMENTAL STUDY

An emerging cell-based strategy in orthopaedics: endothelial progenitor cells

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Abstract
Purpose The purpose of this article was to analyze the results of studies in the literature, which evaluated the use of endothelial progenitor cells (EPCs) as a cell-based tissue engineering strategy.
Methods EPCs have been successfully used in regenerative medicine to augment neovascularization in patients after myocardial infarction and limb ischemia. EPCs' important role as vasculogenic progenitors presents them as a potential source for cell-based therapies to promote bone healing.
Results EPCs have been shown to have prominent effects in promoting bone regeneration in several animal models. Evidence indicates that EPCs promote bone regeneration by stimulating both angiogenesis and osteogenesis through a differentiation process toward endothelial cell lineage and formation of osteoblasts. Moreover, EPCs increase vascularization and osteogenesis by increased secretion of growth factors and cytokines through paracrine mechanisms.
Conclusion EPCs offer the potential to emerge as a new strategy among other cell-based therapies to promote bone regeneration. Further investigations and human trials are required to address current questions with regard to biology and mechanisms of action of EPCs in bone tissue engineering.

Keywords Endothelial progenitor cell (EPC) · Bone tissue engineering · Cell-based therapy · Fracture healing

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Introduction

Bone is a biologically advantaged tissue in that it has the capacity to undergo regeneration as part of a repair process [19]. The unique regenerative process of bone requires coordinated coupling between osteogenesis and angiogenesis [24]. Fracture healing is the most common and recognizable form of bone regeneration [19]. Although regeneration of bone can be completed with minimal scarring, a significant percentage of fractures fail to heal adequately [39]. Non-union or delayed union of fractures, repair of large segmental bone defects after tumor removal, infections, or trauma remains a highly challenging and clinically important problem in orthopaedic surgery with a negative impact on the burden of musculoskeletal diseases. Although new technologies and advances have substantially enhanced fracture healing and surgical outcomes, there is a subset of fractures and related conditions that continue to be deficient in bone repair and culminate in non-union. Consequently, new strategies to optimize bone regeneration are being developed.

Current approaches that aim to promote bone healing such as using bone morphogenetic proteins (BMPs) and mesenchymal stem cells (MSCs) lack angiogenic activity for bone regeneration.

Bone healing and blood supply

As early as 1763, the importance of blood vessels in bone formation was noted: "the origin of bone is the artery carrying the blood and in it the mineral elements" [27]. Vascular ingrowth at the fracture site has a cardinal role in the healing process and regeneration of the bone after a fracture [14, 26]. Inadequate blood supply is a major cause of delayed union or non-union during fracture healing [18]. Previous work has shown that the delayed or non-union rate can be as high as 46% in fracture patients with concomitant vascular injuries [16]. Lu et al. [48] demonstrated that an ischemic insult in the hind limb prior to fracture leads to delayed union or non-union in a mouse model with tibia fracture.

Studies have revealed a spatial relationship between the transformed endothelial cells, reticular cells, polymorphic mesenchymal cells, and osteoblasts within fracture callus and suggested that these cells may be interrelated—that is, the endothelial, reticular, and polymorphic cells may be osteoblast progenitor cells or may lead to the appearance of the osteoblasts in the early callus [9, 10]. Keith [38] suggested that the endothelial cell (EC) was an osteoprogenitor cell. Trueta [70] described the juxtaposition of ECs to osteoblasts and hypothesized that the EC was the osteoblast precursor cell. Other investigators have also reported the intimate association between growing blood vessels and osteoblastic new bone formation [32].

In 1963, Trueta also suggested the existence of a "vascular stimulating factor (VSF)" functioning at sites of bone damage [70]. These early predictions have proven to be remarkably accurate by more recent investigations that showed the positive effects of growth factors and cytokines such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO), respectively, on angiogenesis and bone healing at a fracture site [23, 30, 45].

Endothelial progenitor cells (EPCs)

Angiogenesis (revascularization) is defined as the extension of the present vascular system. The term *angiogenesis* denotes the formation of new blood vessels from pre-existing ones, whereas *vasculogenesis* is the term used for the formation of new blood vessels when there are no pre-existing ones. Vasculogenesis (neovascularization), previously believed to occur only during embryologic development, defines a process in which endothelial precursor cells (angioblasts) migrate and differentiate in response to local cues (such as growth factors and extracellular matrix) to form new blood vessels. Recently, however, it was realized that vasculogenesis can also occur in the adult organism.

In 1997, Asahara et al. [4] reported for the first time that purified hematopoietic progenitor cells expressing endothelial-associated markers (i.e. cluster of differentiation molecule, CD34) from adults can differentiate into an endothelial phenotype, and the authors named these cells as "endothelial progenitor cells (EPCs)."

Although there are various descriptions in the literature with respect to the origin and the surface markers of these cells, EPCs can be defined as bone-marrow-derived precursor cells with the ability to differentiate into endothelial cells and to participate in the formation of new blood vessels. Given the variety of multi-potent cell types that appear to reside within adult bone marrow (BM) (and the common embryologic origins of the vascular and hematopoietic systems), it is highly plausible that BM is the primary source of endothelial progenitors, which can be mobilized to the peripheral circulation and may seed in remote organs and tissues such as liver, spleen, heart, muscle, and adipose tissue [17].

Endothelial progenitor cells have been shown to express various endothelial surface markers such as CD34, VEGFR2, and CD133 and to home to sites of ischemia [31, 71]. On the other hand, hematopoietic stem cell populations also express these surface markers. Hence, using flow cytometry to select the cells with these markers may be affected by hematopoietic contamination. To minimize this

contamination in cell selection, EPCs are cultured and identified using endothelial cell-specific culture media and identification protocols, respectively (Fig. 1a, b). A variable to be considered when performing endothelial differentiation studies is that putative EPCs might only give rise to ECs depending on the exact combination of growth factors to which the cells have been exposed in vitro, and/or depending on the animal model used to assess postnatal vasculogenic activity, and/or the nature, extent, or method of delivery of the angiogenic stimulus applied in vivo.

These important caveats, in what should be the most important criterion to validate EPCness, make the field of EPCs intriguing, but at the same time complicated. Therefore, further efforts should be focused on the development of a standard assay or set of assays that are accessible to all investigators, to specifically define and validate the function of (candidate) EPCs, so that investigators would have a benchmark for comparison and a rationale for the examination and clinical translation of selected cell subsets in targeted clinical disorders [47, 69].

The bottom line is that EPCs can induce the formation of new blood vessels from the pre-existing ones (revascularization) and also in conditions when there are no pre-existing ones (neovascularization) such as during embryonic development [4, 43, 51]. Since the conditions in environments such as a fracture site or segmental bone defect, ischemic limb and myocardium may be variable in terms of existence or lack of blood vessels, and it is plausible to use the word "re-/neovascularization" to describe the effects of EPCs on blood vessel formation in vivo.

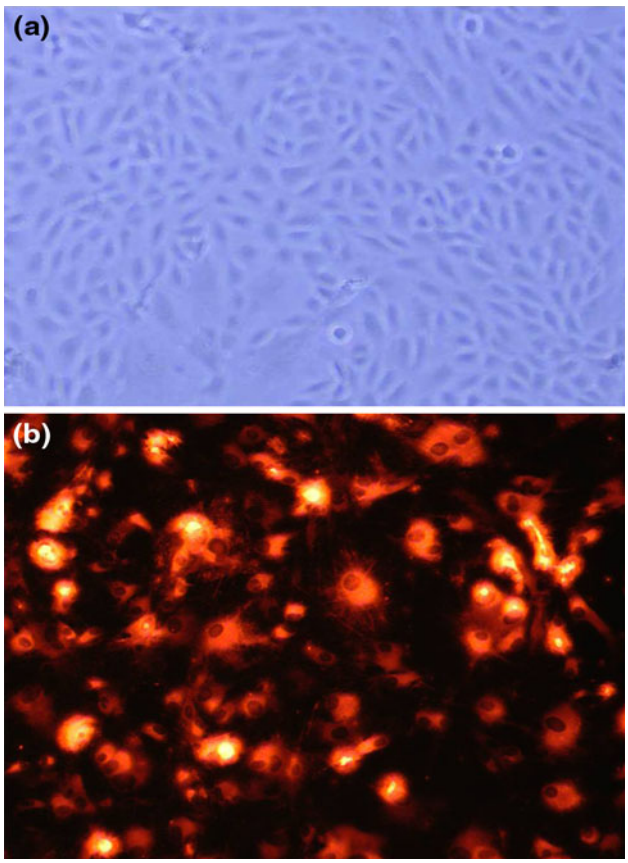

Fig. 1 **a** Characteristic cobblestone pattern of EPCs at day 10 in fibronectin-coated flask (phase contrast microscope ×20 magnification). **b** Phenotype of cultured cells is further characterized according to their ability to uptake Dil-labeled acetylated LDL. The entire cell population is effectively stained positive with Dil-Ac-LDL, which confirms their endothelial lineage. *Note*: View of EPCs after trypsin application; cells not attached to flask (fluorescent microscope ×40 magnification)

EPC mobilization and chemotaxis

The mobilization of stem cells in the BM is determined by the local microenvironment, the so-called stem cell niche, which consists of fibroblasts, osteoblasts, and ECs [54]. Physiologically, ischemia is believed to upregulate mobilizing cytokines such as VEGF or stromal-derived factor (SDF)-1. This in turn hampers the interactions between EPCs and stromal cells via a matrix metalloproteinase-9 (MMP-9)-dependent mechanism [13], and EPCs are mobilized from the BM into the circulation (Fig. 2).

EPC interaction with nitric oxide, VEGF, and erythropoietin

Nitric oxide (NO), known as the endothelium-derived relaxing factor (EDRF), is used by the endothelium of blood vessels to signal the surrounding smooth muscle to relax. This consequently results in vasodilation and increase in blood flow. Endothelial nitric oxide synthase (eNOS) is an enzyme that generates NO in blood vessels and has a role in regulating vascular tone by inhibiting smooth muscle contraction and platelet aggregation. Previous investigations have provided inferential evidence that biological processes modulated by NO extend to include re-/neovascularization, and eNOS activity has an essential role in the formation of new blood vessels [55, 57, 73]. Studies demonstrated that MMP-9, which is required for EPC mobilization from the BM, is activated by NO (Fig. 2). Hence, eNOS deficiency may cause defective mobilization of EPCs from the BM and contributes to the impairment of ischemia-induced re-/neovascularization [3].

Vascular endothelial growth factor is a signal protein that has role in the formation of new blood vessels. While VEGF is primarily known as a vascular-permeability

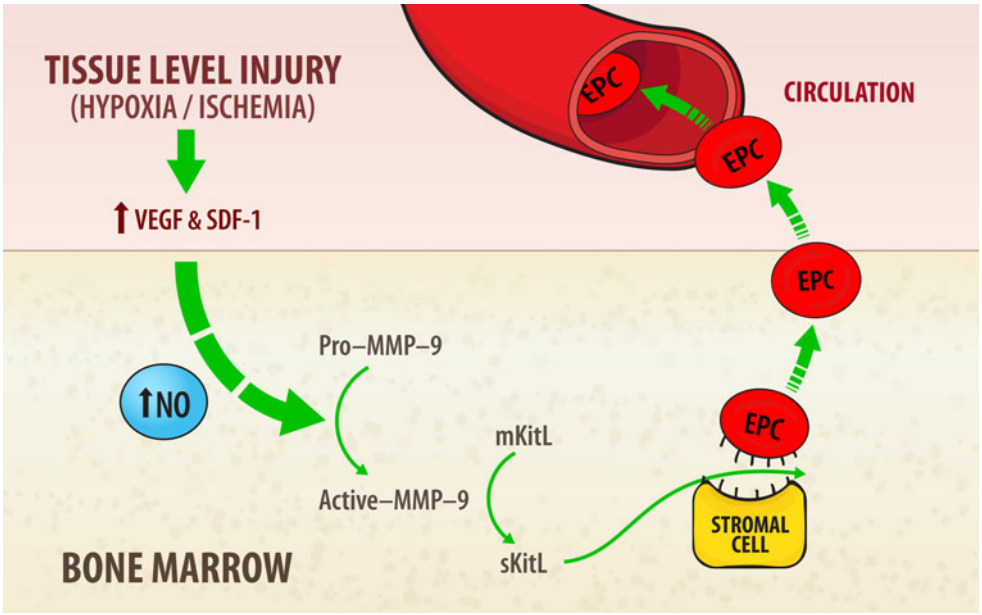

Fig. 2 Simplified schematic representation of endothelial progenitor cell (EPC) mobilization from the bone marrow. Tissue injury and ischemia upregulate VEGF or SDF-1 release into circulation. This activates pro-MMP-9 through an NO-dependent mechanism. Active-MMP-9 causes the release of sKitL from mKitL. sKitL is the ligand for a tyrosine kinase receptor expressed by EPC. After ligand binding, the receptor auto-phosphorylates and this triggers EPC efflux from the bone marrow. sKitL also activates proteinases that breach the adhesive bonds between the EPCs and stromal cells (*VEGF* vascular endothelial growth factor, *SDF-1* stromal-derived factor 1, *NO* nitric oxide, *MMP-9* matrix metalloproteinase-9, *mKitL* membrane-bound Kit Ligand, *sKitL* soluble Kit Ligand)

factor, it has been also shown to secrete endothelial cell mitogens with high-affinity binding sites for endothelial cells. VEGF may promote the proliferation, differentiation, and maturation of and inhibit the apoptosis of vascular endothelial cells. Additionally, it may perform other functions such as inducing migration of vascular smooth muscle cells, promoting the synthesis and secretion of matrix metalloproteinases (MMPs), and inducing chemotactic movement of inflammatory cells, thereby promoting the formation of new capillaries [53]. VEGF plays a crucial role in promoting the differentiation and maturation of EPCs.

Erythropoietin is a glycoprotein hormone that controls erythropoiesis. Studies have shown that EPO has significant mitogenic effects on endothelial cells and enhances angiogenesis in ischemic tissues [15, 28, 36]. Furthermore, both EPO and VEGF share important activities with respect to angiogenesis and exhibit similar angiogenic potential on endothelial cells [33]. In a comparative study, Bahlmann et al. [8] studied the effects of EPO on modulation of functionally active EPCs. The results of this study revealed that treatment with recombinant human erythropoietin (rhEPO) causes a significant mobilization of CD34(+)/CD45(+) circulating progenitor cells in peripheral blood (PB) and increases the number of functionally active EPCs.

EPCs for therapeutic re-/neovascularization

Endothelial progenitor cells' major role in new vessel formation and their ability to proliferate and differentiate into endothelial cells present them as an ideal therapeutic alternative for ex vivo expansion and transplantation into ischemic areas [72]. Studies have shown that infusion of PB-derived EPCs, bone marrow mononuclear cells, or purified CD34+ cells may all demonstrate potential to improve re-/neovascularization and myocardial function in a variety of animal models with an acute infarct [35, 40]. Schuster et al. [63] isolated EPCs from humans treated with recombinant granulocyte colony-stimulating factor. These cells were injected to athymic rats after ligation of the left anterior descending coronary artery. Their results showed that the degree of re-/neovascularization induced in the ischemic myocardium is directly related to the numbers of CD34-positive angioblasts homing to the ischemic site. In parallel with growth of larger-sized capillaries accompanying injection of high concentrations of human EPCs, ischemic rat hearts developed prominent islands of regenerating myocytes around the infarct region.

Trans-endocardial injection of unselected BM cells or EPCs has been associated with enhanced collateral flow and increased capillary density in porcine models of chronic myocardial ischemia [21, 37].

Improvement in tissue perfusion has also been demonstrated following infusion of ex vivo-expanded EPCs and autologous bone marrow mononuclear cells in animal models with peripheral vascular insufficiency [34, 56]. Kalka et al. [34] transplanted human endothelial progenitor cells (hEPCs) to athymic nude mice with hind limb ischemia. Blood flow recovery and capillary density in the ischemic hind limb were markedly improved, and the rate of limb loss was significantly reduced.

In the past 5 years, human trials of EPC therapy for cardiovascular disease have also been conducted and results have demonstrated favorable effects of EPCs on post-infarction myocardial perfusion and left ventricular remodeling [5, 12, 62, 66].

An interesting finding that has been consistent in most of the studies was that the incorporation rate of EPCs in an ischemic tissue model was quite low, or at least not enough to explain the observed increase in re-/neovascularization. The challenge is to illuminate how such a low number of endothelial stem cells can improve re-/neovascularization. One possible explanation is that the efficiency of new blood vessel formation may combine the incorporation of EPCs in newly formed vessels and the release of proangiogenic factors in a paracrine manner [13].

EPC mobilization and bone regeneration

Biological cascades resulting in new vessel formation are initiated following an injury to the vascular or musculoskeletal system. Previous studies have documented the increase in EPC mobilization into PB circulation after vascular trauma and myocardial infarction [25, 65]. Mobilization of EPCs in response to musculoskeletal trauma has been studied by Laing et al. [42] in patients with closed diaphyseal tibial fractures. The authors demonstrated that circulating CD34-positive mononuclear cell levels increased sevenfold by day 3 post-injury. These cells were identified as EPCs (bound UEA-1 and incorporated fluorescent DiI-Ac-LDL intracellularly), which suggests that a systemic provascular response is initiated in response to musculoskeletal trauma. The authors suggested that in addition to an inflammatory response and cytokine release, a competent responsive endothelial cell population is required to obtain angiogenic effects and healing at a fracture site following musculoskeletal trauma.

Lee et al. [43] evaluated the change in the proportion of EPCs during fracture healing and distraction osteogenesis (DO) and investigated whether the mobilized EPCs were transferred to the bone regeneration site to participate in re-/neovascularization. The authors used rodent tibia fracture and DO models in their study. In the fracture model, EPC levels in PB circulation increased approximately sixfold at postfracture day 3 compared with non-operated control animals. In the DO model, the proportion of EPCs increased significantly on post-osteotomy day 3 compared with controls and decreased gradually to baseline levels. Interestingly, in the DO group, the EPC count increased significantly during the distraction and consolidation periods compared with animals where no distraction was performed after the osteotomy. The authors reported that the relative blood flow of the fragment proximal to the osteotomy gap began to increase toward the end of the distraction period and peaked during the consolidation period that was after EPC mobilization induced by the distraction strain. As a part of the study, ex vivo-expanded and tagged EPCs were transplanted via an intravenous route to the DO model animals to assess the distribution of the cells. Transplanted cells were dose-dependently localized primarily in the spleen. This finding was consistent with the findings from previous studies using rat myocardial infarction models in which over 70% of intravenously transplanted EPCs were localized to the spleen [2]. The possible explanation for this preference for the spleen may be that the spleen is the major homing site of immunocompetent cells [2, 29]. Lee et al. [43] noted that "although only a few of transplanted cells were found at the bone regeneration site, a distinct dose-dependent relationship was observed between the number of infused transplanted cells and the number of cells identified at the distraction gap." The authors detected no tagged EPCs in the contralateral non-operated tibia after transplantation. They concluded that the proportion of EPCs increases by signals from bone regeneration sites and this appears to contribute to re-/neovascularization and thus to increase in blood flow during fracture healing and DO in rodent models.

In a study by Matsumoto et al. [52], fluorescence-activated cell sorting (FACS) analysis demonstrated that the frequency of bone marrow (BM) and peripheral blood (PB) EPCs significantly increased postfracture. The EPC-derived re-/neovascularization at the fracture site was confirmed by double immunohistochemistry for CD31 and Sca1 (stem cell antigen-1). The authors showed that EPCs contributing to formation of new blood vessels at the fracture site were specifically derived from BM. Systemic administration of PB green fluorescent protein (GFP)-positive EPCs further confirmed incorporation of the mobilized EPCs into the fracture site for fracture healing. Their findings indicated that fracture may induce mobilization of EPCs from BM to PB and recruitment of the mobilized EPCs into the fracture sites, thereby augmenting re-/neovascularization during the process of bone healing. In their study, the authors utilized a reproducible animal model of femur fracture with severe decrease in local blood flow, which was proven by laser Doppler perfusion imaging. The natural history of this model was found to be

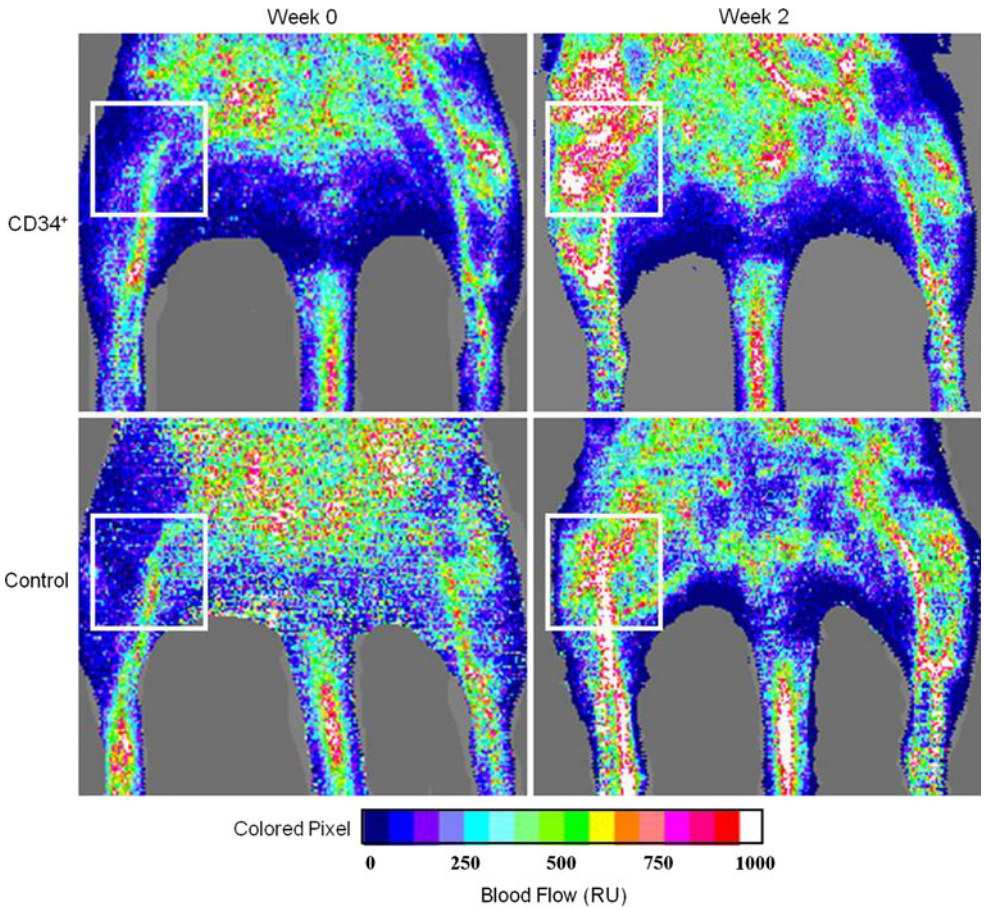

Fig. 3 Laser Doppler perfusion imaging (LDPI) demonstrating blood flow to fracture site in rats with transverse femoral shaft fracture. At week 0 (1 h after fracture), there was no difference in blood flow to the fracture site (*square*) between the animals from CD34+ cell–transplanted group (*upper picture*) and from the control group treated with saline infusion (*lower picture*). At week 2, blood flow to the fracture area (*square*) was remarkably enhanced in the animal from CD34+ cell transplantation group (*upper picture*) compared with control animal (*lower picture*). *Note*: CD34+ cell population includes endothelial progenitor cells

relevant to the clinical situation of the common fracture. However, histological results demonstrated that part of neovascularization at the fracture site is independent of vasculogenesis by BM-derived EPCs, suggesting other mechanisms such as the paracrine effect of the BM-derived EPCs on resident EPCs and ECs.

Local EPC therapy to augment bone regeneration

Previous studies reporting the effects of EPCs in re-/neovascularization and the increase in mobilization of these cells following fractures have started attracting researchers to explore the potential of these cells further in fracture regeneration (Fig. 3).

A pioneering study by Matsumoto et al. [50] investigated the therapeutic potential of systemically administered CD34+ cells on fracture healing in a rodent model. The authors transplanted human peripheral blood CD34+ cells, mononuclear cells (MNCs), or saline to immunodeficient rats with a non-healing femoral fracture. Fracture healing was significantly enhanced in the CD34+ group compared to the MNC and saline groups. Laser Doppler imaging demonstrated that fracture-induced ischemia was significantly recovered in the CD34+ cell–transplanted group compared to the other groups. Transplanted human CD34+ cells were labeled, and their recruitment at the fracture site was confirmed. Reverse transcriptase–polymerase chain reaction (RT-PCR) and immunohistochemical staining at the peri-fracture site confirmed expression of human-specific markers for endothelial cells and osteoblasts 2 weeks after CD34+ cell transplantation. The authors also noted that approximately 20% of human peripheral blood CD34+ cells expressed mRNA for osteocalcin after transplantation to fracture site. These findings may indicate the potential of CD34+ cells for osteogenic and endothelial differentiation, and paracrine secretion of growth factors such as VEGF.

In a rat tibia fracture model, Lee et al. [43] observed that ex vivo-expanded EPCs were collected by the spleen in large quantities compared with those that were homed to the fracture site following intravenous application. Hence, local use of ex vivo-expanded EPCs at a fracture site to enhance new vessel formation and augment bone healing has appeared as a more potent approach compared with intravenous administration of these cells.

Effects of local treatment with ex vivo-expanded EPCs on healing of a critical-sized bone defect have been

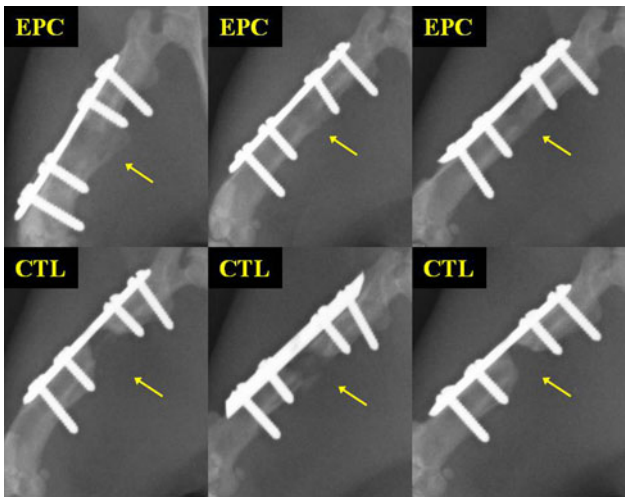

Fig. 4 Radiographs of a segmental defect in rat femur bone taken 10 weeks after local EPC or saline (control) application. Union with bridging callus formation was observed in all the animals in the EPC group (*upper row*) and in none of the animals in the control group (*lower row*)

reported recently. Rozen et al. [61] implanted ex vivo-expanded autologous EPCs into a wedge-shaped gap platform in sheep tibiae and compared the bone regeneration with a control group treated with sham operation. Radiographic and microcomputed tomographic (micro-CT) analysis at 12 weeks after the procedure revealed complete bridging of the gap in six out of seven animals with better parameters of bone formation in the EPC-transplanted group compared with sham-treated animals where the new bone formation was minimal. Histological analysis of the gap tissue at 12 weeks showed dense and massive woven bone formation all throughout the defect in the EPC-transplanted group compared to the control group where the defect was mostly filled with fibrotic scar tissue.

The first study reporting the effects of local EPC therapy on healing of a segmental bone defect was published by Atesok et al. [6]. The authors evaluated the effects of the local use of ex vivo-expanded EPCs on the stimulation of angiogenesis and the promotion of bone healing at a fracture site in a rat femur osteotomy model. They compared the EPC-treated group ($n = 28$) with a control group ($n = 28$), and the animals were killed at 1, 2, 3, and 10 weeks postoperatively. Radiographically, mean scores of the EPC group at 1, 2, and 3 weeks were found to be significantly higher compared with the control group. At 10 weeks, all the animals in the EPC group had complete union (7/7), but in the control group, none achieved union (0/7) (Fig. 4). Micro-CT assessment showed significantly improved parameters of bone healing for the EPC group compared to control group (Fig. 5). Histologically, specimens from the EPC-treated animals had abundant new bone formation compared with controls (Fig. 6). The authors stated that "local EPC therapy significantly

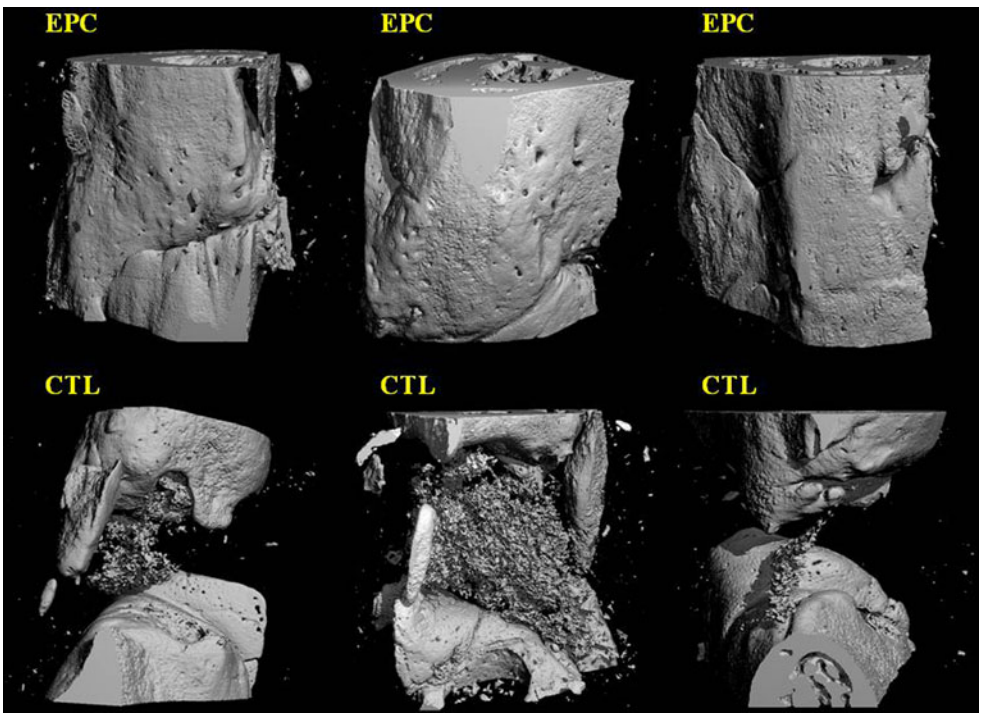

Fig. 5 Micro-CT images of a segmental defect in rat femur bone 10 weeks after local treatment with EPCs or saline (control). Superior bone healing with filling of the entire defect with new bone in the EPC-treated group (*upper row*), compared to insufficient bone formation in the control group (*lower row*)

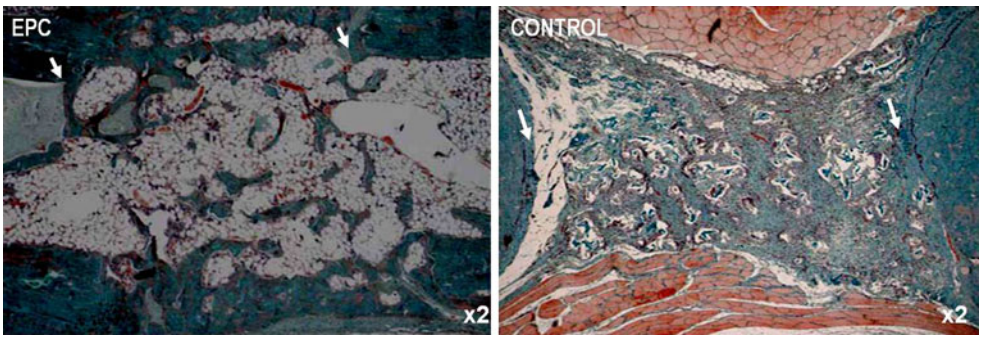

Fig. 6 Histological specimens from a segmental defect in rat femur bone 10 weeks following local application of EPCs or saline (control). EPC group slide (*left*) shows newly formed trabecular bone filling the defect and bridging the osteotomy gap. Control group slide (*right*) shows predominantly fibrotic tissue with patchy areas of ossification and collagen structure (stained *green*) filling the defect. *Arrows* indicate the edges of the osteotomy gap (Masson's Trichrome Staining; ×2 magnification)

enhanced bone regeneration in a segmental bone defect in rat femur." In a similar study, the same research group also reported that local EPC therapy affects biomechanical stability favorably [44].

EPC biology, differentiation, and cellular interactions with MSCs

Although EPC biology and effect mechanisms are to be studied further, there is some evidence indicating that these cells promote bone regeneration by stimulating both re-/neovascularization and osteogenesis through a differentiation process toward an endothelial cell lineage and formation of osteoblasts, respectively [51, 59]. Moreover, EPCs increase re-/neovascularization and osteogenesis by increasing secretion of growth factors and cytokines through paracrine mechanisms.

Endothelial progenitor cells are originated from hematopoietic stem cells, and the origin of osteoblasts is MSCs. Based on this fact, the theory that supports differentiation of EPCs directly into osteoblasts owing to their plasticity appears to be arguable because adult stem cell populations, although exceptionally plastic, are somewhat restricted to the production of cells from the tissue of origin. In the last few years, various reports have challenged this central dogma by demonstrating that adult stem cells, under certain conditions, generate cell types beside those in the tissue of origin. These cell behaviors have been termed as "cross-differentiation." Such reports have generated excitement as well as scepticism in the field of stem cell biology, as the concept of "cross-differentiation" defies the developmental biology principle that lineage restriction is imparted during morphogenesis. Resolution of the "cross-differentiation" controversy requires extensive future studies and will obviously have a major impact on the current definitions of stem cells [67]. It can be speculated that current terminology describing stem cell populations based on the tissue of origin will become unfeasible if the theory that adult stem cells have the ability to change fate can be scientifically proven.

Recent reports also suggest that EPCs derived from PB contribute to osteogenic differentiation by MSCs in vitro and that MSCs support the proliferation of EPCs and stabilize the formed cellular networks [20]. Aguirre et al. [1] investigated the physical and biochemical interactions between bone marrow endothelial progenitor cells (BM-EPCs) and MSCs in an in vitro coculture system. Their data suggested that cross talk occurs between BM-EPCs and MSCs through paracrine and direct cell contact mechanisms leading to modulation of the angiogenic response. These interactions between MSCs and EPCs appear to further strengthen the capacity of EPCs to enhance bone healing at a fracture site.

Seebach et al. [64] evaluated the effects of EPCs alone or in combination with MSCs on early neovascularization and bone healing in a critically sized defect using a rat model. The authors suggested that there is a synergistic effect between EPCs and MSCs and that the initial stage of neovascularization by EPCs is considered to be crucial for complete bone regeneration.

Further assessment of the interactions between EPCs, MSCs and other cellular and protein elements at a fracture site can illuminate actual potential of EPCs. Furthermore, this may provide valuable information with regard to validity of using EPCs with other cell-based therapy options simultaneously.

Current obstacles

Bone tissue engineering is a novel way to repair osseous lesions with cell-free devices or scaffolds seeded with cells. Despite extensive evidence from proof-of-principle studies, bone tissue engineering, particularly the use of scaffolds seeded with cells, has not translated to clinical practice.

Similar to other cell-based therapies, EPCs also need to be studied further to justify their use as a valuable tissue engineering strategy in orthopedic surgery. Much of the research to date involves in vitro and animal models that do not replicate potential clinical applications. Major problem areas include determination of the correct cell source and number [11]. The correct source to isolate EPCs and their safety in vivo still needs to be explored. Moreover, determination of the minimum number of EPCs required to obtain maximum effect per unit of bone defect is essential to delineate future investigations on isolation, culturing, and transplantation techniques.

Another point worth discussing is the need to choose the right scaffold to transplant EPCs into the host. An ideal scaffold material must provide for the ease of implantation, bone-like mechanical stiffness, cell attachment, proliferation and migration, nutrient–waste exchange, vascularization and ingrowth of new bone commensurate with biomaterial degradation, and in vivo integration [11]. However, evidence to date does not include data with regard to biocompatibility of the EPCs with currently available scaffolds.

Future perspectives and clinical rationale

Vascularization remains one of the main obstacles that needs to be overcome before large tissue-engineered constructs can be useful in clinical settings. Inability to provide sufficient blood supply in the initial phase after implantation can lead to improper cell integration or cell death in tissue-engineered constructs [60].

One of the challenges in orthopaedic tissue engineering is to accelerate the integration of implants in the body. Previously it was shown that therapeutic application of VEGF offers potential to enhance blood supply and healing through revascularization around an engineered implant in a regulated manner [58]. EPC therapy can be a promising strategy to increase the success of tissue healing and implant integration since revascularization is the key process in osseointegration of implants.

Endothelial progenitor cell therapy can be also used for the acceleration of graft revascularization and enhancement of tendon-bone osseointegration following anterior cruciate ligament (ACL) reconstruction. This may hasten revitalization of the tendon graft and integration to the bone, which eventually allows earlier and aggressive rehabilitation and speedy return to sports and daily activity. Matsumoto et al. [49] recently demonstrated that CD34- and CD146-expressing vascular cells exist in human ACL tissues, have a potential for multi-lineage differentiation, and are recruited to the rupture site to participate in the intrinsic healing of injured ACL.

In a rodent model, Tei et al. [68] studied the effects of locally transplanted human peripheral blood CD34+ cells on the healing of medial collateral ligament (MCL) injury. Macroscopic, histological, and biomechanical assessments showed significantly enhanced ligament healing in a CD34+ cell transplantation group compared with a control group. The authors suggested that "local transplantation of circulating human CD34+ cells may augment the ligament healing process by promoting a favorable environment through neovascularization."

Avascular necrosis (AVN) appears as another major clinical problem to be addressed. Although it was previously shown that transplanted MSCs can migrate into and survive in necrotic femoral heads, reversal of necrosis or preservation of function has yet to be studied [22, 46]. EPCs may present as a new frontier in AVN research with their angiogenic and osteogenic features.

Based on the promising results from ex vivo and animal model EPC studies, clinical trials have been started by Matsumoto, Asahara, Kuroda, and colleagues. As a pilot case from a phase I/IIA clinical trial, Kuroda et al. [41] reported the results of transplantation of autologous peripheral blood CD34+ cells, the hematopoietic/EPC-enriched population, in a patient with non-union of a tibia fracture. Clinical and radiological healing of the fracture was achieved at 12 weeks after the cell therapy with bone grafting, and no serious short-term complications were encountered.

Clinical applications using EPCs as a cell-based strategy directed to accelerate re-/neovascularization at a fracture site and to improve bone healing could result in a reduced incidence of delayed or non-union. Consequently, this will bring benefits in terms of health care costs and improved quality of life.

Conclusion

Local use of EPCs emerges as a promising cell-based therapy to promote bone regeneration at a fracture site. EPCs, with their unique features, such as ability to differentiate into endothelial cells and participate in the formation of new blood vessels and high plasticity, may offer therapeutic alternatives for repair of cartilage tissue, treatment of AVN, osteochondral defects, augmentation of tendon-to-bone healing, and ligament repair [7].

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