

## Bioengineering of Bone Marrow Endothelial Cells, the Prospect of Bone Microenvironment as Therapeutic Measures

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### ABSTRACT

The bone marrow endothelial-vascular niche facilitates the proliferation, differentiation, and survival of hematopoietic stem cells (HSCs) and non-hematopoietic stem cells such as osteoblast/osteoclast and mesenchymal stem cells. This fertile microenvironment is a safe place not only for healthy cells and malignant hematopoietic cells but also for a tumor the spreads to the bone, endeavor protection to chemotherapeutic agents by common pathways. This occurs by secretion of the SDF-1 from arteriole-sinusoidal associated stromal cells (CAR, LepR<sup>+</sup> nest-MS-C), vascular endothelial cadherin (CD144), E-selectin, VEGFR, and angiopoietin-1 (Ang-1) contribute to the maintenance of the HSCs. Research in tissue in engineering and regenerative medicine has continued to show development toward the potential treatment of chemotherapy-resistant malignancy and structural genetic disease. Therefore, an experiment focusing on extracting endothelial stem cells and reprogramming with CRISPR/cas9 gene editing can be used to evaluate all disorders from reconstruction and regeneration of blood-related genetic disease and drug-resistant malignancy.

**Keywords:** Bone marrow microenvironment (BMM), Bone marrow endothelial cells (BMECs), Endothelial-vascular niche, Hematopoietic stem cells (HSCs)

### INTRODUCTION

In the bone marrow microenvironment, the endothelial vascular niche provides the vascular network structure and release molecules that are involved in the recruitment of hematopoietic stem cells (HSCs) and non-hematopoietic stem cells such as endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs), fibroblast, osteoblasts/osteoclasts, macrophages, T lymphocytes, and mast cells [1,2]. The term endothelial -vascular niche indicates the proximity to the bone marrow vascular rich in blood vessels specialized in mobilization, proliferation, and differentiation of the HSCs-the bone marrow microenvironment consists of arteriolar vascular place and sinusoidal endothelial slot. The sinusoidal endothelial vessel supplies oxygen, nutrient, and growth factors to the HSCs to promote proliferation and differentiation. It also encourages the mobilization and homing of cytokines through chemokines such as stromal cell factor (SCF), CSF2, and Flt3 [3,4]. The endothelial cells within the sinusoidal vessel express the adhesion molecules such as vascular endothelial growth factor receptor (VEGFR), selectin molecules [E-Selectin (CD62E), P-selectin (CD62P)], and intracellular adhesion molecule-1 (ICAM-1). These adhesion molecules provide cell-cell interaction and extracellular matrix that

play a role in homing, regulation of apoptosis, immune response to inflammation, and lymphocyte proliferation and differentiation [5-7]. The arteriolar niche unsheathed exclusively by NG2 pericyte (Nest-GFP<sup>+</sup> NG2<sup>+</sup>) distinct from the sinusoidal vessel. The SDF-1 secreted from arteriole-sinusoidal associated stromal cells (CAR, LepR<sup>+</sup> nest-MS-C), vascular endothelial cadherin (CD144), and VCAM-1 and angiopoietin-1 (Ang-1) contribute to the maintenance of the HSCs [3,4] (**Figure 1**).

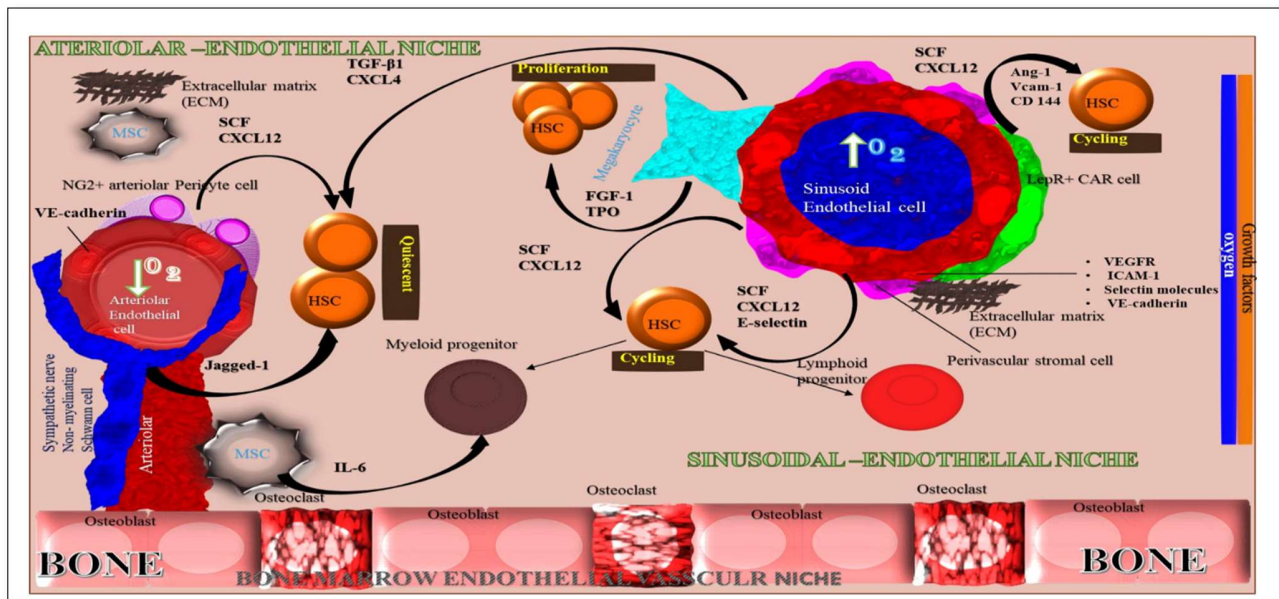
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**Figure 1.** The bone marrow endothelial-vascular niche.

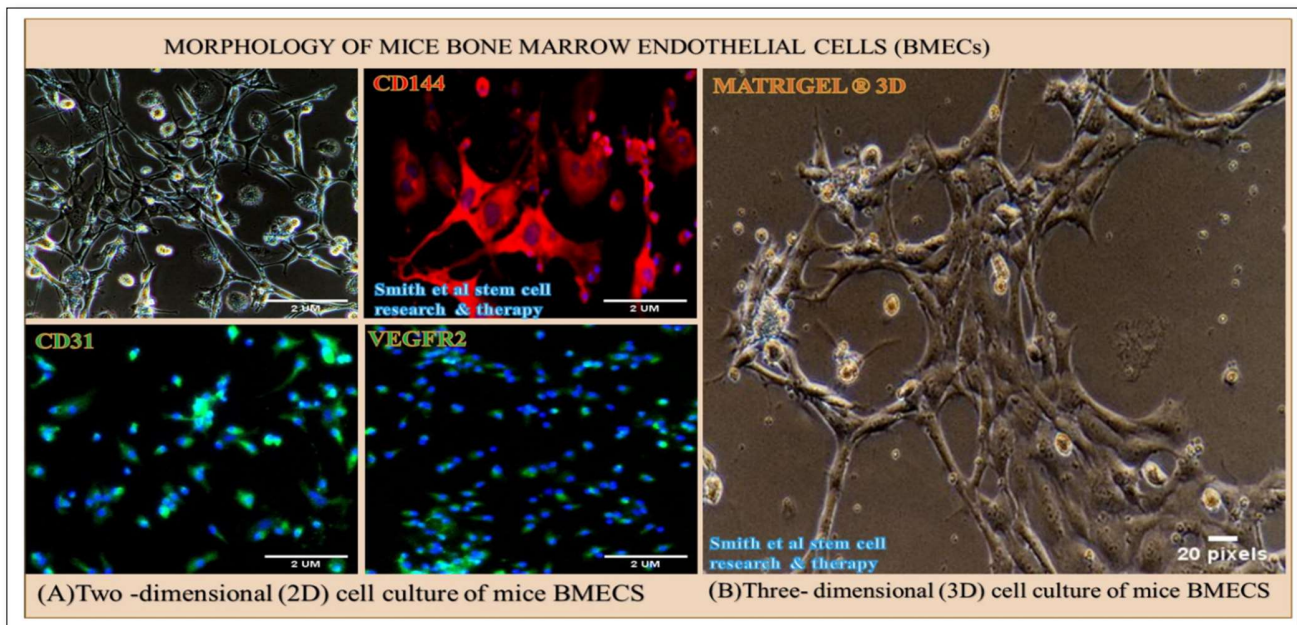
MSC: Mesenchymal Stem Cell; SCF: Stromal Cell Factor; IL-6: Interleukin-6; TGF- $\beta$ 1: Transforming Growth Factor-Beta -1; E-selectin: Endothelial Selecting Molecule; VEGFR: Vascular Endothelial Growth Factor Receptor; VE-cadherin: Vascular Endothelial Cadherin; FGF-1: Fibroblast Grow Factor -1; CXCL4: Chemokine Ligand 4; ICAM-1: Intracellular Adhesion Molecule-1

The illustration of the arteriolar (NG2<sup>+</sup> arteriolar pericyte cells) and sinusoid vascular (LepR<sup>+</sup>CAR cells) niche in the bone marrow microenvironment. It shows the relation between the hematopoietic stem cells (HSCs) and non-hematopoietic cells such as osteoblast, osteoclast, extracellular matrix, mesenchymal stem cells, and sympathetic non-myelinating Schwann cell. It also described the secreting cytokines such as SCF, CXCL12, CXCL4, and E-selectin that promote recovery of the HSCs.

#### Morphology and bioengineering of bone marrow endothelial cells

In the sinusoid- endothelial vascular niche of the bone marrow niche, endothelial cells play a critical role in establishing the sinusoid vessel's vascular network. This characteristic morphology of the endothelial cells is associated with proliferation, differentiation, elongation, and assembly to form cord-like vessels/ capillary lumen called vascular networks [8-10]. These vascular structures line the interior of the sinusoidal vessels and maintain tissue hemostasis by delivering oxygen, growth factors, and nutrients and eliminating waste products. The angiogenesis

of the endothelial cells involves the activation of resting cells by vascular endothelial cells growth factor (VEGF) and fibroblast growth factor (FGF). With the degradation of the basement membrane, the tip of the cells starts to increase and elongate to form the capillary spindles, which then assembly to form the perusable lumen [11,12]. The use of two-dimensional (2D)-cell culture of bone marrow endothelial cells will be difficult to maintain the in-vivo characteristic of the endothelial cells within the vascular niche due to the monolayer attached of the cells to the plastic dish. The formation of an endothelial vascular network in 2D-monolayer cell culture and evaluation of angiogenesis has been challenging over the years. However, the introduction of the three-dimensional (3D) engineered tissues research can now elicit the angiogenesis function of these cells supplement with growth factors and their response to inflammatory stimuli [13,14]. Successful application of these methods, either separately or in combination, is expected to improve the therapeutic strategy and develop functional cells for regenerative endothelial stem cells (**Figure 2**).



**Figure 2.** The morphology of bone marrow endothelial cells in two-dimensional (2D) and three-dimensional (3D) cell cultures. (A) 2D cell culture morphology of mice bone marrow endothelial cells stained with endothelial-specific markers; vascular endothelial cadherin molecule (CD144), Platelet endothelial cell adhesion molecule (PECAM-1/CD31), and VEGFR: vascular endothelial growth factor receptor. (B) 3D cell culture of bone marrow endothelial cells with the characteristic capillary lumen and vascular network of the bone marrow endothelial cells.

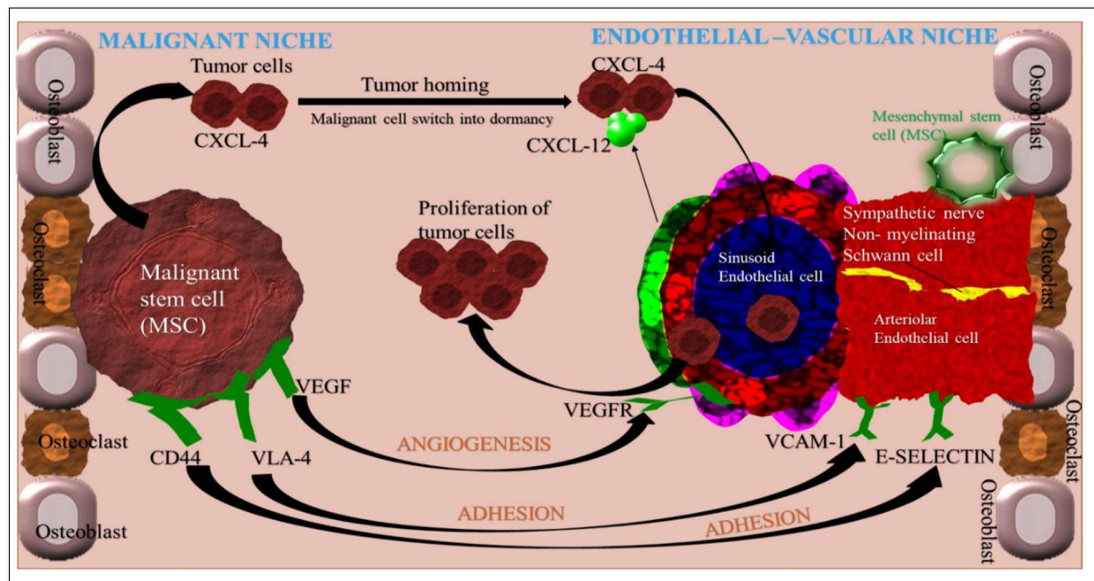
### The bone marrow niche is the home of normal and malignant stem cells

The bone marrow microenvironment is fertile for primary and secondary tumors such as osteosarcoma, leukemia, lymphoma, multiple myeloma, breast cancer, and prostate cancers. Structural and architectural cues tightly regulate the endothelial-vascular niche. The vascular wall barriers supply oxygen, growth factors, nutrients, and drug delivery [15,16]. High doses of chemotherapeutic agents may cause damage to the endothelial-vascular barrier wall, increase permeability, altered perfusion and release normal hematopoietic stem cells into the periphery. Recently, the relapse of myeloid malignancy or lymphoma due to engraftment of the tumor in the bone marrow niche. These dynamic changes include the expression of adhesion molecules involved in cell migration and interaction of the extracellular matrix. The invasion of the malignant cells into the bone marrow environment may hijack the hemostatic mechanism of the endothelial-vascular network, take refuge within the bone marrow niche during chemotherapy, and eventually contribute to the disease's relapse [2,5,17]. The existence of the malignant stem cells in the bone marrow microenvironment is due to the interactions of CXCR4/CXCL12(SDF-1), very late antigen-4 (VLA-4)/ vascular

adhesion molecule (VCAM-1), vascular endothelial cell growth factor (VEGF)/ vascular endothelial growth factor receptor (VEGFR) and CD44/ E-selectin respectively. These molecular pathways support the survival, proliferation, adhesion, and angiogenesis of malignant stem cells. Therefore, targeting these molecular pathways by newer chemotherapeutic agents or recombinant immunotherapy may serve as a new therapeutic measure to improve the prognosis of patients with the recurrence of hematological malignancies [18-22] (Figure 3).

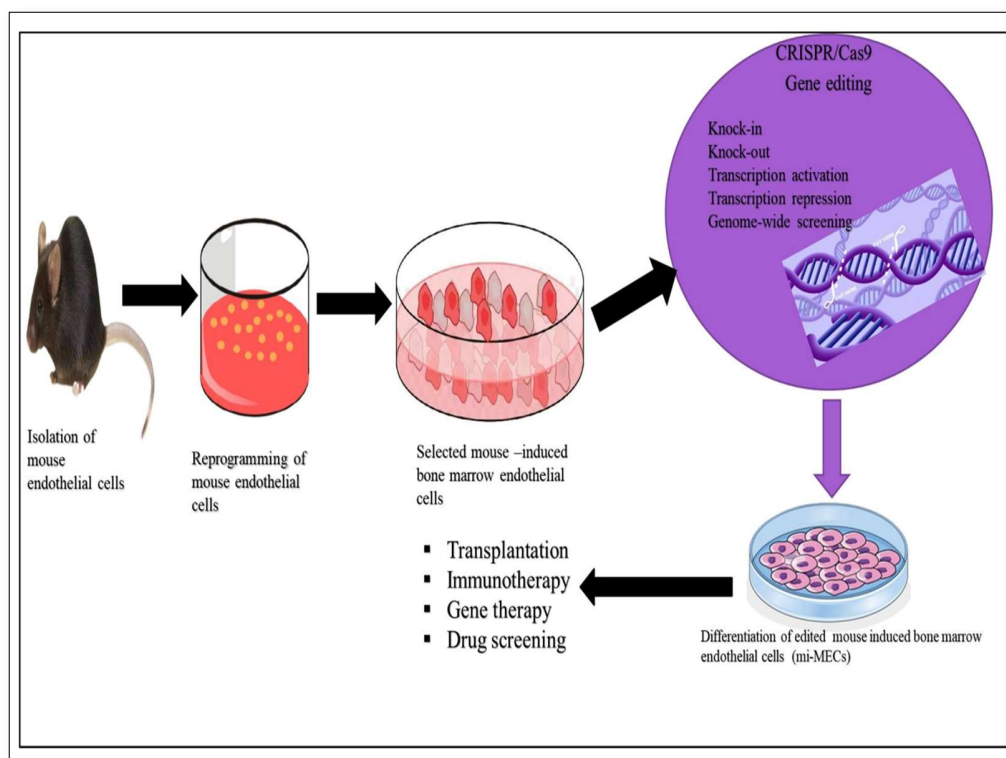
### The prospect of bone marrow endothelial cells by using CRISPR/cas9 genomic editing

Recently, the development of clustered regularly interspaced short palindromic repeats (CRISPR) molecular scissors that cut DNA for effective gene editing. These technological tools allow the researcher to altered the potential DNA sequence and modify gene function. The CRISPR methodology has been employed to participate in human endothelial cells by mutating cultured endothelial cells to produce a homogenous population. The generation of bone marrow endothelial cells could be for conventional gene therapy, drug discovery and transplantation for hematological genetic disease, and chemotherapeutic resistant malignancies [23,24] (Figure 4).



**Figure 3.** The molecular mechanism of malignant stem cells and the endothelial vascular niche. It illustrates the invasion of the malignant cells into the bone marrow microenvironment and duping of the molecular mechanisms by the cancer cells via; tumor homing and chemoattractant, angiogenesis, adhesion, and eventual proliferation of the tumor.

*THC: Tumor Homing and Chemoattractant; CXCL4: Chemokine Ligand 4; CXCL12 SDF-1; Stromal-Derived Factor -12, Angiogenesis; VEGF/VEGFR: VEGFR: Vascular Endothelial Growth Factor; VEGFR: Vascular Endothelial Growth Factor Receptor, Adhesion; VLA-4/ Vascular Adhesion Molecule (VCAM-1), and CD44/ E-selectin*



**Figure 4.** The prospect of bone marrow endothelial cells with CRISPR/cas9 genomic editing. It described the process of genomic editing after isolating bone marrow endothelial cells from the mice, followed by differentiation for further application.

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