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Review

Macrophage regulation in vascularization upon regeneration and repair of tissue injury and engineered organ transplantation

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ABSTRACT

Tissue engineering and regenerative medicine are effective strategies for the treatment of damaged tissues and end-stage organ failure. Damaged tissue regeneration and organ transplantation require blood vessel reconstruction to facilitate tissue remodeling, the bottleneck for application research in this field. Immune cells are heavily involved in coordinating neovascularization, in which macrophage aggregation is a key factor in angiogenesis and arteriogenesis. Previous studies have promoted tissue vascularization by regulating macrophages; however, the mechanisms underlying macrophage-mediated vascularization remain nebulous. Studies on material-based regulation have primarily been observational and lack systematic and targeted research. Macrophages from different sources exhibit different phenotypes or functions in tissues, such as peripheral blood monocytes and tissue-resident macrophages, with each exhibiting complicated mechanisms for promoting tissue injury and graft remodeling. Therefore, in this review, we discuss the role of different tissue-resident macrophages and circulating monocytes in vascularization during injured tissue regeneration and graft remodeling and summarize the current strategies for the use of biomaterials to regulate macrophages and promote the vascularization of injured tissues and during organ transplantation. A better understanding of these mechanisms will facilitate future tissue engineering research that promotes vascularization by regulating macrophage reactions.

1. Introduction

Tissue repair and regeneration are essential biological processes. Chronic or dysregulated wound healing response may impair normal tissue function and lead to organ failure and death. To satisfy the need for organ replacement, tissue engineering has been used to create tissues capable of replacing damaged organs. Vascularization is important for tissue damage repair and function of transplanted organs as the lumen of a blood vessel is essential for providing blood [1]. In naturally intact tissues and organs, the microvascular system delivers sufficient nutrients and oxygen while removing waste and carbon dioxide. Angiogenesis is therefore an important component of physiologic tissue repair, and reperfusion of damaged tissue is an essential step in the healing process. Following injury, microvasculature is disrupted, leading to fluid accumulation, inflammation, and hypoxia, potentially resulting in impaired or chronic wound healing [2]. Vascularization remains a major challenge in tissue engineering. All organisms growing beyond the oxygen diffusion limit critically depend on functional vasculature for sur-

Abbreviations: AMPK, AMP-activated protein kinase; Arg-1, arginase-1; CCL, chemokine ligand; CCR, chemokine receptor; CD62L, L-selectin; CX3CR1, CX3C chemokine receptor 1; CXCR4, SDF-1 α receptor; ECM, extracellular matrix; fbR, foreign body reaction; FGF, fibroblast growth factor; GAG, glycosaminoglycan; GCA, giant cell arteritis; HA, hyaluronic acid; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; iNOS, nitric oxide synthase; Ly6C, lymphocyte antigen 6C; M2e, M2 endothelial; M-CSF, macrophage colony stimulating factor; MCP-1, monocyte chemoattractant protein-1; MDP, multidomain peptide; MI, myocardial infarction; miRNA, microRNA; MMP, matrix metalloproteinase; MΦ, inactive macrophage; NF-κB, nuclear factor κB; NO, nitric oxide; NR4A1, nuclear receptor subfamily 4 group A member 1; PDGF, platelet-derived growth factor; PEG, polyethylene glycol; SDF1, stromal cell-derived factor 1; SF, silk fibroin; siRNA, small interfering RNA; SIS, small intestinal submucosa; TEVG, tissue-engineered vascular graft; TGFβ1, transforming growth factor-β1; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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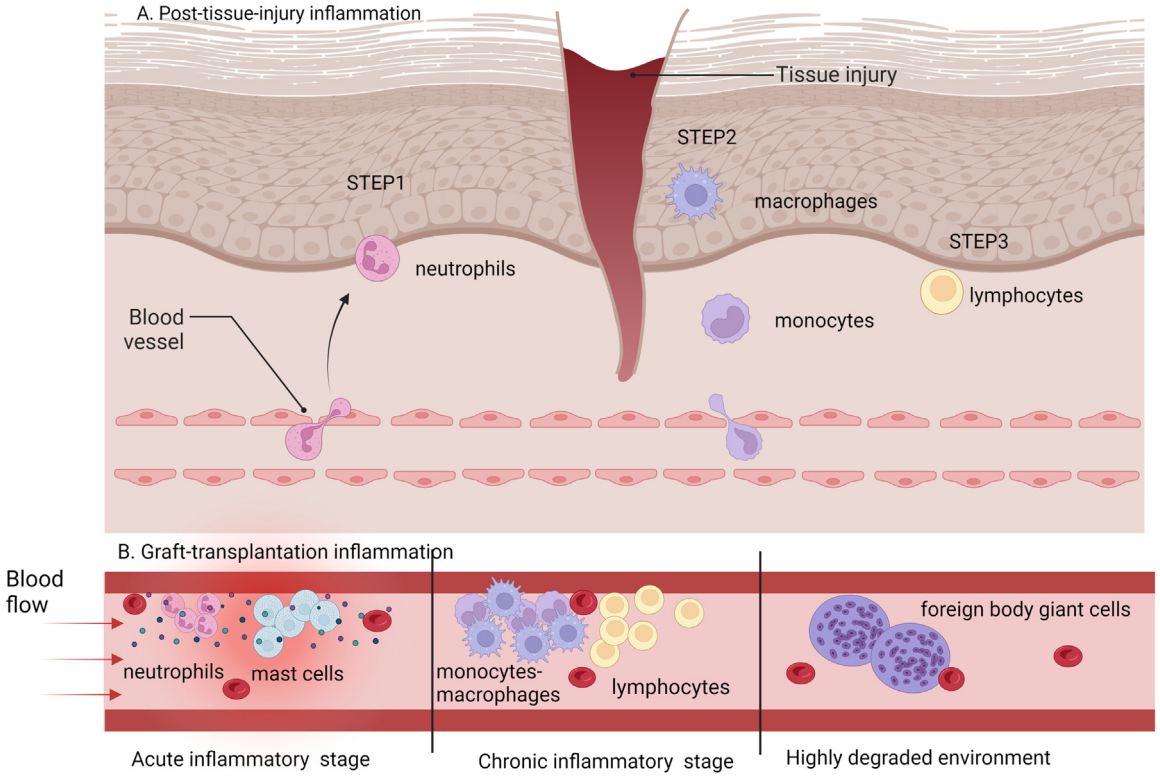

Fig. 1. Inflammation following tissue injury and organ transplantation. (A) Inflammation is initiated after tissue injury. Step 1: the neutrophil influx initiates an inflammatory response; step 2: monocytes infiltrate and differentiate into macrophages; step 3: innate immune cell signaling recruits lymphocytes to the site of injury, exacerbating the innate immune response. (B) Inflammation occurs following organ transplantation. Acute inflammatory stage: proteins in plasma adsorbed to the surface of the graft form a sparse matrix containing innate immune cells, primarily neutrophils, mast cells, and their granule products involved in acute inflammation. Chronic inflammatory stage: Acute inflammation is rapidly resolved within one week, followed by a chronic inflammatory response characterized by monocytes and lymphocytes. Highly degradable environment: macrophage fusion after chronic inflammation produces foreign body giant cells, mediating a highly degraded environment. The illustration was created with BioRender.com.

vival [3]. Therefore, tissue or organ replacement with a size exceeding 400 μm, as is characteristic of thick, engineered tissues, must be vascularized to construct perfusable vascular structures [4].

Revascularization of tissue injury and vascularization of transplanted organs involve a robust immune response [5]. Indeed, the immune system plays a key role in tissue injury repair (Fig. 1). Post-tissue-injury inflammation, triggered by hypoxia and inflammatory cytokines that activate endothelial cells, begins with an influx of neutrophils; subsequently, monocytes infiltrate and differentiate into macrophages. Signals from these innate immune cells, in turn, recruit lymphocytes to the injury site, which further influences the innate immune response. This phase, which naturally subsides in acute cases, is characterized by the transformation of inflammatory macrophages at the site of injury into pro-repair cells [6]. The spatiotemporal pattern of immune cell infiltration post-organ transplantation differs from that of tissue injury. Following organ or tissue-engineered graft implantation, plasma proteins are rapidly adsorbed to the graft surface and form a sparse stroma containing innate immune cells [7]. The acute inflammatory response in the graft primarily involves neutrophils, mast cells, and their granular products and is usually subsided within one week, followed by initiation of a chronic inflammatory response. Chronic inflammation, followed by macrophage fusion, produces foreign body giant cells that facilitate highly degraded environments, leading to a remodeling phase. Therefore, macrophages play different roles within each phase of tissue damage repair and organ transplantation [8].

Moreover, macrophages can mediate neoangiogenesis and the repair of damaged vascular tissue [9]. Notably, tissue-engineered grafts undergo angiogenesis and fusion (anastomosis) of the native vascular network under certain conditions following the acute, chronic, and remodeling phases post-implantation. Due to their low off-target effects and reversible epigenetic programming characteristics, macrophages are important regulatory targets that promote injured tissue repair and graft vascularization.

2. Origin and typing of macrophages in vascularization

Macrophages can maintain *in vivo* homeostasis and resist pathogenicity. As important mediators of tissue vascularization under physiological and pathological conditions, macrophages play different roles in development, response to infection, and tissue injury and repair. Radiolabeling studies have proposed bone marrow-derived circulating monocytes as the source of tissue macrophages. Macrophages were thought to be derived entirely from hematopoietic stem cell-derived monocytes in the bone marrow and released into the peripheral blood circulation, and the expression of chemokine receptor 2 (CCR2) was required for the entry of monocytes from the bone marrow into the blood (Fig. 2A) [5]. Blood monocytes include classical and non-classical subtypes, with two CCR2-based subpopulations that could subsequently differentiate according to L-selectin (CD62L) and CX3C chemokine receptor 1 (CX3CR1) expression. Monocyte cells with CCR2+CD62L+CX3CR1^{low} expression are defined as classical monocytes with pro-inflammatory characteristics that circulate in the blood for several days before leaving the circulation through hemocytosis (diapedesis) to be recruited to tissues in inflammatory conditions. Conversely, monocytes with CCR2–CD62L–CX3CR1^{high} expression are non-classical monocytes with anti-inflammatory properties and complementary to the tissue-resident macrophage population and remain in circulation and migrate along the endothelium, mediat-

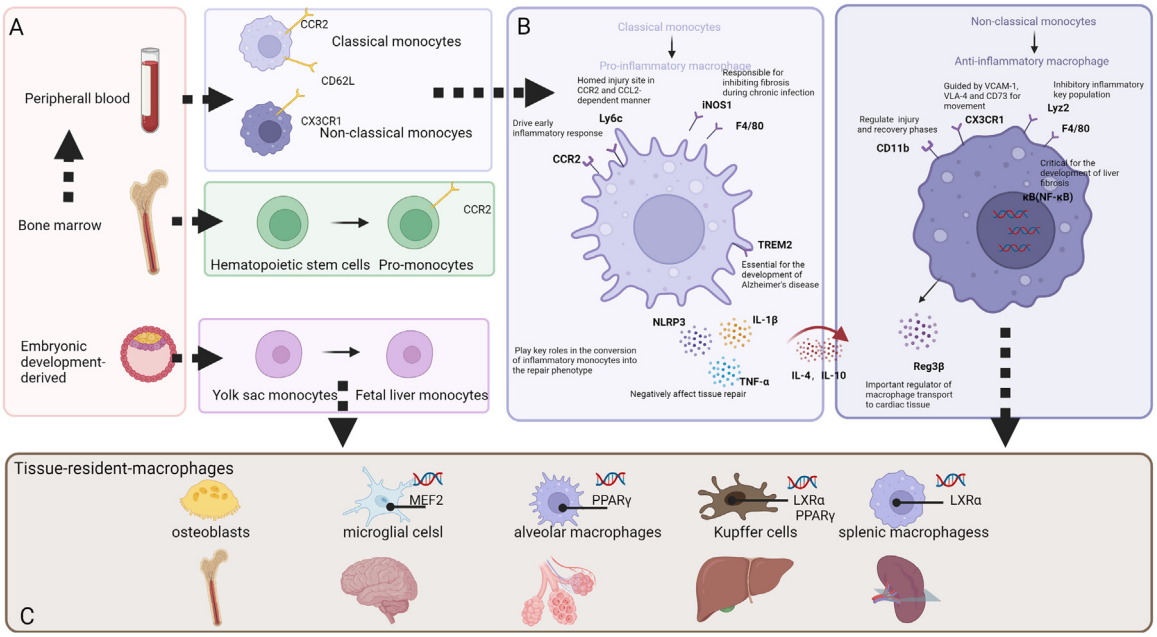

Fig. 2. Past and present lives of macrophages. (A) Macrophages are derived from hematopoietic stem cell-derived monocytes in the bone marrow and released into the peripheral blood circulation. The entry of monocytes from the bone marrow into the bloodstream requires the expression of chemokine receptor 2 (CCR2). Transferred monocytes include both classic and non-classical subtypes. Monocyte cells with CCR2+CD62L+CX3CR1^{low} expression are defined as classical monocytes, whereas those with CCR2−CD62L−CX3CR1^{high} expression are non-classical monocytes, which comprise supplemental tissue-resident macrophage populations. Macrophages are also derived from progenitor cells in the yolk sac and function as tissue-resident macrophages. (B) The various functions of pro-inflammatory and anti-inflammatory macrophages are defined by the specific patterns of marker expression. Pro-inflammatory macrophages include those with CCR2+, Ly6c^{high}CX3CR1^{low}, arginase-1 (Arg1)^{high}F4/80+CD11b+Lyz^{low}, TREM2+, and CX3CR1+ expression, while anti-inflammatory macrophages include those with CD11b+ and either Ly6c^{low}CX3CR1^{high} or Lyz2 ^{high}F4/80+CD11b+ expression. (C) Tissue-resident macrophages from different tissues express specific transcription factors such as GATA-6 in peritoneal macrophages, MEF2 in microglia, LXRα in Kupffer cells and splenic macrophages, and PPARγ in alveolar and splenic macrophages. The illustration was created with BioRender.com.

ing wound healing and vascular patrol (Fig. 2B). Lymphocyte antigen 6C (Ly6C) is another specific marker of monocyte macrophages.

CCR2+CD62L+CX3CR1^{low}-expressing Ly6C+ monocytes mediate cell infiltration into inflamed tissues and produce pro-inflammatory cytokines and chemokines [9].

Subsequent lineage-tracing studies have demonstrated that macrophages originate from monocytes and progenitor cells in the yolk sac and comprise tissue-resident macrophages that replicate in specific tissues prior to the development of monocyte-derived macrophages [10]. Such tissue-resident macrophages can survive for long periods in most tissues, are self-renewing, and can maintain homeostasis without the contribution of circulating monocytes. Tissue-resident macrophages are defined by the unique microenvironment in which they reside. Transcriptomic analysis of resident macrophages from different tissues identified tissue-of-residence-specific transcription factors, such as GATA-6 in peritoneal macrophages, MEF2 in microglia, LXRα in Kupffer cells and splenic macrophages, and PPARγ in alveolar and splenic macrophages (Fig. 2C). Tissue-resident and monocyte-derived macrophages play different roles in tissue injury and repair. Typically, monocyte-derived macrophages show a strong inflammatory response and may undergo apoptosis or self-renewal. Alternatively, tissue-resident macrophages scavenge apoptotic cells, proteins, and phospholipids, remove or respond to toxins, particles, and pathogens within the local microenvironment, maintain themselves through local proliferation, and may die or expand through self-renewal to repopulate ecological niches [10].

It has been previously hypothesized that tissue-resident macrophages are the first class of macrophages to respond to trauma, subsequently recruiting monocytes to differentiate into (inactivated) macrophages (MΦ). Following tissue injury, the number of tissue-resident macrophages significantly exceeds that of inflammatory monocytes recruited from the bone marrow via chemokine gradients and various adhesion molecules [8]. Recruited and resident macrophages are influenced by the cytokines or growth factors released in the local tissue microenvironment to alter their phenotype and function, a process known as MΦ macrophage polarization. Activated MΦ macrophages can be divided into two distinct subpopulations, namely the classically activated M1 type and the alternatively activated M2 type. M1 macrophages are anti-angiogenic, whereas M2 macrophages, including the M2a, M2b, and M2c subpopulations, are pro-angiogenic. Specific macrophage types, termed M2 endothelial (M2e) cells, which express key endothelial cell genes and produce nitric oxide (NO), may also exist [11].

Improving the inflammatory environment by regulating the activation status of macrophages is an effective strategy to regulate angiogenesis. Increasing evidence indicates that different monocyte and macrophage populations play different roles in tissue repair, inflammatory response, and fibrosis and that inflammatory monocytes and tissue-resident macrophages often have opposing functions in tissue injury repair. CD11b+ macrophages regulate the injury and recovery phases of tissue repair, whereas CCR2+ monocyte-derived cells drive the early inflammatory response post-injury. The pro-inflammatory Ly6c^{high} CX3CR1^{low}-monocyte population is recruited to the tissue injury site in a CCR2 and chemokine ligand 2 (CCL2)-dependent manner, whereas VCAM-1, VLA-4, and CD73 guide the movement of the reparative Ly6c^{low} CX3CR1^{high} monocyte population. Lyz2^{high} F4/80+CD11b+ mature tissue macrophages are the key inhibitory inflammatory population, whereas Lyz^{low} F4/80+CD11b+ monocytes expressing high arginase-1 (Arg-1) inhibit fibrosis during chronic infection. Regenerative islet-derived 3β (Reg3β) regulates macrophage transport to cardiac tissue following injury, while interleukin 4 (IL-4), IL-10, and phagocytosis mediate the conversion of inflammatory monocytes into cells of the

repair phenotype (Fig. 2B). Monocytes can also have pro-inflammatory and pro-repair functions, suggesting that *in situ* conversion, rather than recruitment of pro-repair Ly6C-subsets, is essential in some scenarios.

Alternatively, pro-inflammatory macrophages exacerbate tissue damage, such as those expressing trigger receptors on myeloid cells 2 (TREM2), which are involved in the development of Alzheimer's disease. CX3CR1+ macrophages are associated with axonal injury, and those producing NLR family pyrin domain containing 3 (NLRP3), IL-1β, and tumor necrosis factor α (TNF-α) negatively affect tissue repair. Macrophages promote fibrosis, with continued activation or sustained recruitment of M(IL-4)-like cells contributing to the development of pathological fibrosis. Production and activation of the pro-fibrotic cytokine transforming growth factor-β1 (TGF-β1) are also associated with pro-fibrotic macrophage activity; hepatic macrophage nuclear factor κB (NF-κB) is critical for the development of liver fibrosis, while macrophage-derived matrix metalloproteinase-12 (MMP12) is essential for the development of IL-13 and TGF-β1-driven fibrosis models.

Some cells exhibit anti-inflammatory and anti-fibrotic properties, which are critical for the resolution of most wound healing responses. The macrophage-produced immunomodulatory cytokine IL-10 has an anti-inflammatory function. Activating transcription factor 3 (ATF3) is a target for downregulating Toll-like receptor (TLR)-induced pro-inflammatory cytokine production in macrophages. The transcription factor nuclear receptor subfamily 4 group A member 1 (NR4A1) regulates the severity of autoimmune encephalitis by inhibiting autocrine norepinephrine production in macrophages, whereas NR3B1 regulates the anti-inflammatory macrophage function by controlling macrophage metabolic reprogramming. Anti-inflammatory macrophages also regulate the development and maintenance of IL-10 and TGF-β1-producing regulatory T (Treg) cells, which contribute to resolving tissue-damaging inflammatory responses in various tissues, and M (IL-4) macrophages slow the progression of fibrosis by suppressing local CD4+ T cell responses and reducing extracellular matrix (ECM)-based myofibroblast production [8].

3. Macrophages in vascularization during damaged tissue repair and regeneration

The vascular system is the first functional organ to develop in the embryo and is essential for nutrient and oxygen supply [11,12]. Angiogenesis, the biological process of growing new blood vessels on the original vascular structure, is driven by angiogenic proteins, including angiogenic growth factors, chemokines, and extracellular matrix proteins, particularly vascular endothelial growth factor (VEGF) [13]. Vascular growth or angiogenesis involves a series of complex events, including degradation of the basement membrane, endothelial cell migration and proliferation, tube formation, anastomosis of newly formed blood vessels, and stabilization of supporting cells (such as pericytes) [14]. Angiogenesis is crucial for physiological (*e.g.*, tissue regeneration) and pathological (*e.g.*, tumor growth) processes and thus has considerable clinical relevance. In particular, a reduction in wound macrophages leads to a sharp reduction in angiogenesis [15], whereas the addition of exogenous macrophages promotes angiogenesis [16], consistent with the role of macrophages as key regulators of healing.

3.1. Mechanisms of macrophage promotion of vascularization in tissue injury

3.1.1. Signaling pathways

Vessels regulate the differentiation and maturation of macrophages from recruited monocytes through Notch signaling, thereby promoting arterial formation and tissue repair [17]. Meanwhile, macrophage maturation during ischemia is controlled by the Notch ligand Dll1 expressed in vascular endothelial cells, which requires macrophages to transmit typical Notch signals through the recombination signal binding protein for immunoglobulin kappa J region (RBPJ) and inhibit the fate of inflammatory macrophages (Fig. 3).

AMP-activated protein kinase (AMPK) signaling is also required for angiogenesis [18]. AMPK is an energy and redox sensor activated in response to various cellular stresses. AMPKα1 in macrophages promotes collateral remodeling and arteriogenesis in mice [19]. Following femoral artery ligation, global AMPKα1 knockout mice showed significantly reduced blood flow recovery and impaired collateral arteriolar remodeling. Furthermore, macrophage-specific AMPKα1 knockout mice showed similar injury. AMPK directly phosphorylates IκB kinase α (IKKα) and promotes NF-κB-dependent growth factor activity. By controlling the production of monocyte–macrophage TGF, platelet-derived growth factor (PDGF) subunit B, fibroblast growth factor 2 (FGF2), and VEGF through the NF-κB signaling pathway, AMPKα1 plays an important role in regulating adult arteriogenesis and collateral circulation formation (Fig. 3).

Moreover, insulin-like growth factor (IGF) signaling induces coronary endothelial cell migration [20] and may mediate the pro-angiogenesis function of embryonic-derived macrophages (Fig. 3) [21]. Specifically, blocking the ability of CCR2–embryonic macrophages to stimulate coronary endothelial cell tubulation and migration upon inhibition of the IGF1 receptor *in vitro* implicates IGF ligands as potentially relevant pro-angiogenic signals (Table 1).

3.1.2. Factor secretion

VEGF-A recruitment by monocytes/macrophages induces inflammatory neoangiogenesis by providing or amplifying signals necessary for pathological angiogenesis and lymphangiogenesis [22]. Macrophages secrete paracrine factors, such as VEGF-A, which promotes hypothalamic and lymphangiogenesis by binding to VEGFR receptor 2 (VEGFR-2), and VEGF-C and VEGF-D, which promote lymphangiogenesis by binding VEGFR-3 [23,24]. Additionally, macrophages express VEGFR-1 and VEGFR-3, which mediate myeloid cell chemotaxis, perpetuating the inflammatory submembranous and lymphangiogenic response (*i.e.*, immune amplification) [22]. IL-10-activated macrophages are important in inflammatory corneal neovascularization [9]. Furthermore, CCR2-deficient mice showed reduced corneal neovascularization concomitant with reduced macrophage infiltration, whereas CX3CR1-deficient mice showed increased macrophage infiltration, as evidenced by corneal neovascularization (Fig. 3) [25]. Deletion of the chemokine receptors CCR2 and CX3CR1 has protective effects on thrombotic stroke and can attenuate the infarction size; meanwhile, CX3CR1 knock-out mice showed increased revascularization at 7 days post-stroke, suggesting that the absence of the CX3CR1 receptor has beneficial effects on the revascularization of the infarction and promotes the development of small vessels [26].

Monocytes promote embryonic vasculogenesis by depositing a VEGF-rich migratory body. A population of highly migratory cells on the chorioallantoic membrane capable of forming migratory bodies in large numbers have been identified as monocytes, the depletion of which impaired capillary formation, suggesting that these cells are important for angiogenesis [27] (Table 2).

3.1.3. Macrophage subsets

Macrophages have distinct functions in response to environmental cues. Classically activated M1 macrophages have pro-inflammatory functions, are polarized by lipopolysaccharide, TNF-α, and cytokines such as interferon-γ (IFN-γ) or granulocyte-macrophage colony-stimulating factor (GM-CSF), and exhibit potent effector (phagocytic) functions against pathogens and cancer cells [28,29].

M1 macrophages secrete pro-inflammatory cytokines, such as IL-6, IL-12, IL-23, and TNF-α, which in turn recruit and activate leukocytes during injury. In contrast, IL-4 and IL-10 stimulate the differentiation of macrophages into the M2 subtype, which exerts anti-inflammatory effects and secretes anti-inflammatory factors, such as TGF-β and IL-10 [30] (Fig. 3). M2 macrophages highly express Arg-1 and anti-

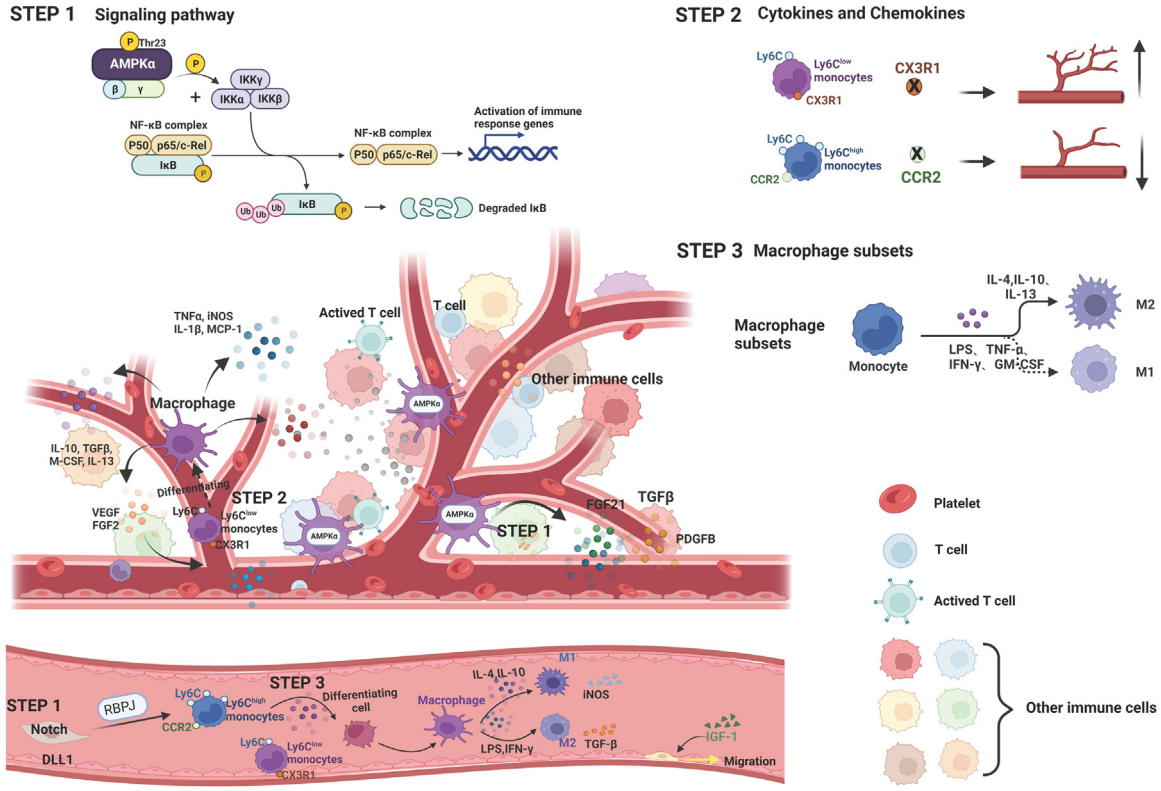

Fig. 3. Mechanisms underlying macrophage promotion of vascularization in tissue injury. Step 1: AMPKα1 in macrophages promotes collateral arteriolar remodeling. AMPKα1 promotes the production of growth factors such as TGF-β by directly phosphorylating inhibitor kappa B kinase alpha (IKKα) at threonine 23 (Thr23). This results in the production of the NF-κB-dependent growth factors PDGFB, FGF2, FGF21, and VEGF, which regulate the formation of arterial and collateral circulation. In addition, blood vessels can control the differentiation and maturation of macrophages from recruited monocytes through Notch signaling, which in turn promotes arteriogenesis and tissue repair. Under the control of Dll1, a Notch ligand expressed in vascular endothelial cells, macrophages transmit canonical Notch signaling through RBPJ to promote macrophage maturation. Concurrently, insulin-like growth factor (IGF) signaling stimulates endothelial cell migration and promotes angiogenesis. Step 2: CCR2 deficiency decreases neovascularization. In contrast, corneal neovascularization is increased in CX3CR1-deficient mice. Step 3: Macrophages exhibit different functions when triggered by vascularization signals and can be divided into two main subsets, the classically activated M1 type or the alternatively activated M2 type. M1 macrophages produce pro-inflammatory cytokines, including TNF-α and oxide synthase (iNOS), which promote the recruitment and activation of white blood cells during injury, whereas M2 macrophages produce anti-inflammatory factors, such as IL-10 and TGF-β, which are thought to be anti-angiogenic. The illustration was created with BioRender.com.

Table 1
Macrophages promote vascularization through different signaling pathways.

Step1 Signaling pathway		
Pathway	Bound ligand or target	Mechanism of action
Notch	Dll1	Macrophage maturation is controlled by Notch ligand Dll1 expressed in vascular endothelial cells and requires macrophage canonical Notch signaling via Rbpj. Rbpj mediates the maturation of ly6chi monocytes to macrophages and promotes arteriogenesis and ischemic tissue repair.
AMPK	IKKα	AMPK regulates NF-κB activation through phosphorylation of threonine 23 on IKKα, an IκB kinase, which in turn enables macrophages to secrete growth factors and promotes interactions between macrophages and SMC/ endothelial cells, leading to arteriogenesis and angiogenesis.
IGF	IGF ligands	IGF signaling induces coronary endothelial cell migration. IGF1 and IGF2 are sufficient to stimulate coronary artery endothelial tube formation and mediate coronary artery remodeling *in vitro*.

inflammatory molecules, such as Fizz1, CD163, CD206, and transglutaminase 2 (TGM2), as well as the chemokine CCL18 [31]. Further-subtyped M2a macrophages are induced by IL-4 or IL-13, whereas M2b macrophages are activated by immune complexes and TLR or IL-1 receptor agonists. M2c cells are stimulated by IL-10 and glucocorticoids [32].

Macrophages drive the vascularization of tissue engineering scaffolds. Indeed, M2 macrophages, including the subsets M2a and M2c, promote angiogenesis and tissue regeneration, while M1 macrophages are anti-angiogenic, although these classifications remain controversial. Contrary studies have suggested that M1 and M2c macrophages induce endothelial cell sprouting; M2a macrophages promote anastomosis, and the three activated macrophage phenotypes, namely M1, M2a, and M2c, support angiogenesis. M1 macrophages promote sprouting of blood vessels via secretion of VEGF, bFGF, IL8, RANTES, and TNFalpha; M2a macrophages may recruit pericytes via secretion of PDGF-BB to stabilize the formed vasculature. M2c macrophages support angiogenesis by increasing vascular remodeling, given their high levels of production of MMP9; M2f macrophages are stimulated by phagocytosis of apoptotic cells (efferocytosis) and secrete anti-inflammatory mediators. TGFB1 is involved in endothelial cross-talk with support cells to regulate vessel stabilization [14,33].

Table 2
Cytokines and chemokines involved in vascularization and their mechanisms of action.

Step 2 Cytokines and Chemokines		
Cytokines and Chemokines	Binding receptor or source cell	Mechanistic explanation
VEGF-A	VEGFR1 and VEGFR2	VEGF-A indirectly stimulates angiogenesis by binding to VEGFR1 and recruiting monocytes/macrophages, which in turn secrete VEGF-C and/or VEGF-D at the site of injury. VEGF-A may also amplify angiogenic responses by recruiting VEGFR1-positive hematopoietic progenitor cells to the affected site and promoting their differentiation into vascular endothelium.
CCR2 or CX3CR1	CCL2 or CX3CL1	CCR2 deficiency reduces macrophage infiltration and neovascularization. CX3CR1 deficiency also reduces macrophage infiltration, however enhances neovascularization. CX3CL1 can induce CX3CR1-positive macrophages to express antiangiogenic molecules such as thrombospondin (TSP)−1 but not vascular endothelial growth factor (VEGF), while CCL2 can induce CX3CR1-negative (probably CCR2 positive) macrophages to express VEGF but not TSP-1
VEGFA and CXCL12	Monocytes	Migrasomes, generated by monocytes, can induce angiogenesis. Monocytes, as a forerunner of angiogenesis, create a favorable microenvironment for angiogenesis before the capillary formation by depositing migrasomes enriched in VEGFA and CXCL12.

Table 3
Macrophages of diverse phenotypes drive the vascularization of injured tissue.

Step3 Macrophage subsets			
Phenotype	Stimulation	Subsets	Biological pathways of macrophage phenotypes involved in angiogenesis
M1 macrophages	LPS, IFN-γ, GM-CSF	M1	M1 macrophages express genes involved in the initiation of angiogenesis, including those that are chemotactic for endothelial cells: VEGF, basic fibroblast growth factor (FGF2), IL8, and CCL5/RANTES.
M2 macrophages	IL-4, IL-13	M2a	M2a macrophages may help to support angiogenesis by recruiting pericytes and regulating the signaling of M1 macrophages. M2a macrophages express and secrete high levels of PDGFB, which recruits pericytes and mesenchymal stem cells to stabilize the formed blood vessels.
	IL-10	M2c	M2c macrophages promote sprouting *in vitro* and secrete high levels of MMP9, a potent stimulator of angiogenesis that contributes to vascular remodeling. M2c macrophages produce osteopontin, a known immunomodulator that enhances VEGF expression in ECs, and promotes proliferation, migration, and tube formation of ECs *in vitro*.
	Apoptotic HL-60 cells	M2f	M2f macrophages are stimulated by phagocytosis of apoptotic cells (efferocytosis) and secrete anti-inflammatory mediators. M2f macrophages upregulate TGFB1, which is implicated in endothelial cross-talk with support cells to modulate vessel stabilization and has been shown to promote EC migration *in vitro* and vessel formation *in vivo*.

The controversy regarding the roles of M1 and M2 macrophages in regulating angiogenesis may be attributed to the temporal effect of macrophage phenotype on vascularization. Short-term presence of M1 macrophages (1 day) has been shown to promote angiogenesis, whereas long-term presence (3 days) resulted in vascular regression, suggesting that the difference in timing is a major contributor to these conflicting findings. Meanwhile, vascular regression is an essential component of healthy angiogenesis; hence, M1 macrophages may support angiogenesis by changing their behavior over time. Sequential addition of M1 and M2a phenotypes may enhance the vascular network formation (Table 3) [14].

In summary, the mechanisms by which macrophages promote the vascularization function of injured tissues include, but are not limited to, signaling pathways, secretion of related factors, and differentiation into different subpopulations, which are summarized as follows:

1. Blood vessels regulate macrophage differentiation and maturation from recruited monocytes via Notch signaling, which in turn promotes arteriogenesis and tissue repair.

2. AMPKα1 in macrophages promotes collateral remodeling and arteriogenesis in mice.

3. IGF signaling is a potent stimulator of coronary endothelial cell migration.

4. VEGF-A recruitment of monocytes/macrophages induces inflammatory neovascularization via supplying/amplifying signals essential for pathological hemangiogenesis.

5. CCR2 and CX3CR1 deficiencies reduce macrophage infiltration; however, CX3CL1 and CCL2 may have opposite effects on macrophages by acting on different receptors during angiogenesis.

6. Monocytes create a favorable microenvironment for angiogenesis by depositing migrators rich in VEGFA and CXCL12.

7. Macrophages with different phenotypes support angiogenesis; M1 and M2c macrophages induce endothelial cell germination, while M2a macrophages promote anastomosis. In addition, angiogenesis and vascularization of injured tissues require a coordinated effort of M1 and M2 macrophages.

These biological pathways highlight the need for in-depth studies investigating the effects of macrophages on angiogenesis and tissue regeneration processes.

3.2. Examples of macrophages promoting tissue regeneration

Early studies on the function of macrophages in wound repair focused on their role as scavenger cells, including phagocytosis of cell debris, invading organisms, neutrophils, and other apoptotic cells following tissue injury. However, recent research, including single-cell RNA sequencing analysis [34], has clarified that monocytes and macrophages play a more complex role in the mechanisms of fibrosis and tissue regeneration, as well as in tissue repair [8].

3.2.1. Neonatal cardiac regeneration

Macrophages facilitate angiogenesis and regeneration in the neonatal mouse heart. Cardiac regeneration and neovascularization after myocardial infarction (MI) are dependent on neonatal macrophages (Fig. 4A–E). Specifically, in the absence of macrophages, neonatal mice lose the ability to regenerate the myocardium and develop fibrotic scars similar to those in aged animals post-infarction, with reduced cardiac function and angiogenesis. Immunophenotypes and gene expression profiles of regenerating and non-regenerating cardiac macrophages suggest that regenerating macrophages have a unique polarized phenotype and secrete numerous soluble factors that promote the formation of new myocardium [35]. Therefore, therapeutic modulation of

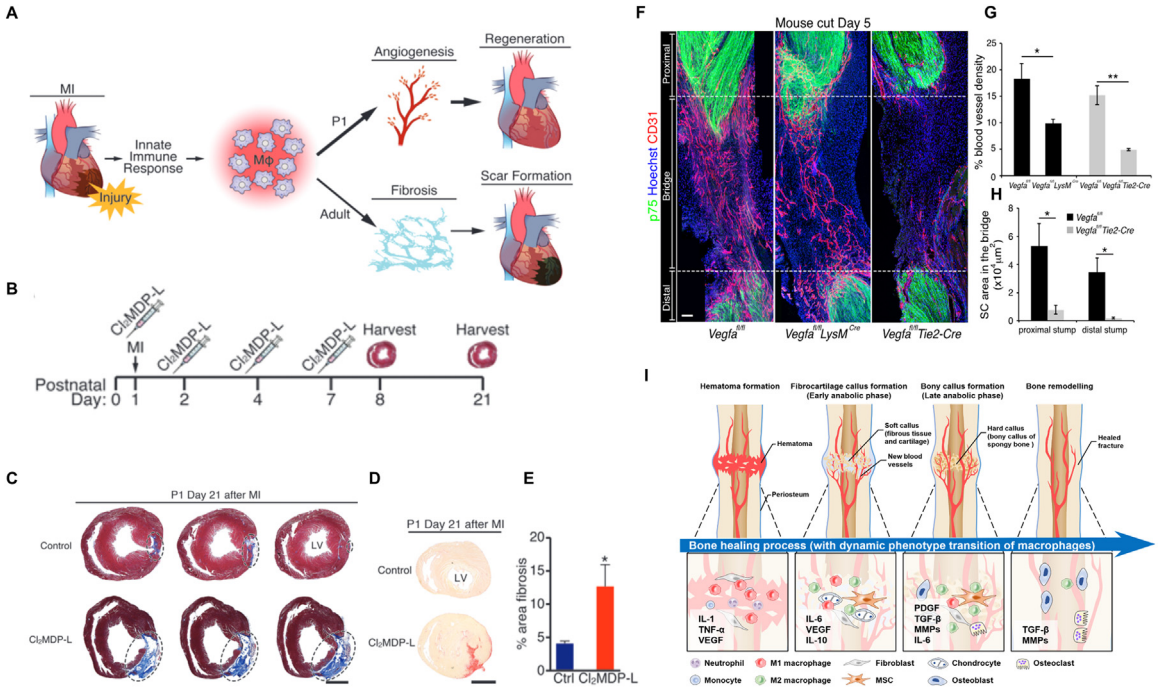

Fig. 4. Macrophage promotion of vascularization. (A) Schematic illustrating the dual roles of macrophages in the process of regeneration following myocardial infarction (MI) (as in postnatal day 1 [P1] mice) or scar formation (as in adult mice). Following MI, the innate immune response to cardiac injury involves the activation and recruitment of cardiac macrophages, which facilitate the removal of cellular debris and secretion of soluble factors. In P1 mice, macrophages promote regenerative processes, such as angiogenesis, while mediating fibrosis and scar formation in adult mice. (B) Experimental strategy to deplete monocytes/macrophages using liposomal clodronate (Cl2MDP-L) following MI in P1 mice. (C–E) At 21 days post-MI, the myocardium of control mice was completely regenerated from the ligation site to the apex. (C) Masson trichrome staining images of mouse hearts 21 days post-MI, showing collagen staining throughout the infarct area to the apex in the Cl2MDP-L-treated group. Sirius red staining shows that the area of fibrotic scar formation in the Cl2MDP-L group was significantly higher than that in the control group. This indicates that regeneration depends on monocytes/macrophages. Scale bar: 1 mm. Reprinted with permission from Aurora et al. [35]. (F) Representative images of longitudinal sections of injured sciatic nerves in mice, examined using immunostaining on day 5 post-transection, immunostained to detect endothelial cells (CD31+, red) and Schwann cells (p75NTR+, green). Inactivation of *Vegfa* in macrophages inhibits the vascularization of nerve bridges following nerve transection. Scale bar: 50 μm. (G) Quantification of the ratio of CD31+ area/bridge area in (F). The results indicate that vascularization of the neural bridge was significantly reduced in the mutant animals. (H) Quantification of the area of (F) microglial influx into both ends of the neural bridge. Reprinted with permission from Cattin et al. [38]. (I) Illustration of a femoral fracture as a representative injury to show the typical fracture healing process and the associated biological events, cellular activities, and cytokines secreted by macrophages involved in these cascade stages. Graphs show mean value ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$. Reprinted with permission from Niu et al. [39].

monocytes/macrophages may promote the regeneration of mammalian hearts.

3.2.2. Peripheral nerve regeneration

A complex dialogue between macrophages and other cell types is required for peripheral nerve regeneration. The peripheral nervous system has an extraordinary ability to regenerate, such as repairing completely severed nerves through the collective migration of nerve cells to guide regenerating axons across the “bridge” formed by the new tissue.

Macrophages secrete cytokines, trigger growth factor synthesis by non-neuronal cells in nerves, and produce factors that promote Schwann cell migration and axonal regeneration [36,37]. Specifically, macrophage-induced polarized blood vessels can guide Schwann cells to migrate and repair a severed sciatic nerve, highlighting their role in directing peripheral nerve regeneration. The multicellular repair process is initiated by hypoxia and selectively sensed by macrophages within the bridge, which induces polarization of blood vessels through VEGF-A secretion, thereby alleviating hypoxia (Fig. 4F–H). Simultaneously, blood vessels provide a guide or “track” for Schwann cell growth, and regenerating axons follow Schwann cells across the bridge to reconnect the nerve [38]. Therefore, VEGF-α produced by macrophages is essential for nerve regeneration [8].

3.2.3. Bone regeneration

Macrophages have three immunomodulatory functions during bone regeneration, namely scavengers, mediators, and guides [39]. As scavengers, macrophages play a phagocytic role during the four stages of bone healing, namely inflammation, soft callus formation (primary anabolism), hard callus formation (late anabolism), and remodeling. Macrophages also mediate cell mobilization, angiogenesis, and matrix remodeling through the release of a variety of paracrine cytokines (Fig. 4I). During the early stage of bone healing, pro-inflammatory cytokines, such as IL-1, IL-6, TNF, and macrophage colony-stimulating factor-1 (M-CSF-1), produce a regenerative habitat [40,41]. However, prolonged pro-inflammatory responses may delay healing, necessitating M2 macrophages, as they secrete growth factors such as PDGF, VEGFs, and various enzymes that accelerate angiogenesis and fracture healing [42–44]. Finally, macrophages directly drive the osteogenic differentiation of osteoprogenitors and bone repair.

3.2.4. Negative role of macrophages in tissue regeneration

Monocytes and macrophages produce various mediators that regulate local tissue progenitor cell renewal and function, which is essential for tissue regeneration. For example, M1-like macrophages release numerous pro-inflammatory exosomes (M1-Exos) post-MI; in turn, M1-Exos exert antiangiogenic effects, exacerbate MI damage, and exhibit a highly expressed pro-inflammatory miRNA, namely miR-155, which inhibits angiogenesis by downregulating target genes in endothelial cells [45]. A population of monocyte-derived IL-10-producing macrophages plays a novel role in neuroprotection and progenitor cell renewal in the damaged adult mouse retina [46]. Moreover, IL-10 switches muscle macrophages from an M1 pro-inflammatory phenotype to an M2 muscle regeneration-promoting phenotype, a shift necessary for proper muscle growth and regeneration [47].

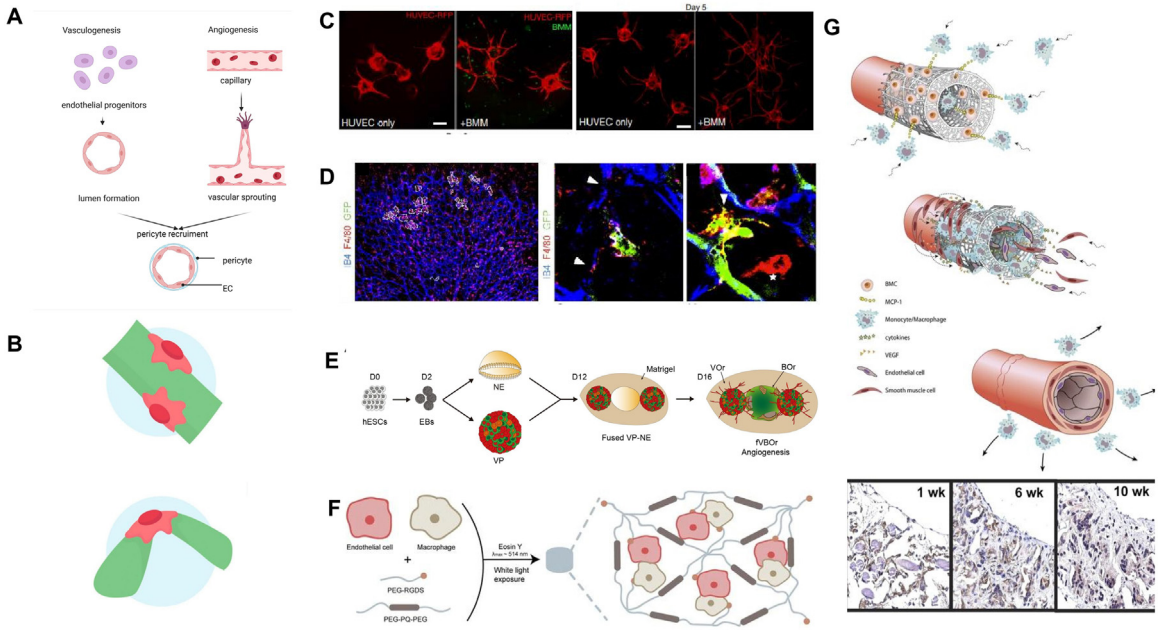

Fig. 5. Macrophages in vascularization of tissue-engineered organ grafts. (A) In the process of natural vascular network formation, angiogenesis comprises the differentiation of angioblasts into endothelial cells and their proliferation within the vascular tissue to form a primitive vascular network. Angiogenesis thus constitutes the germination of new blood vessels from an existing network of blood vessels. (B) Two types of interactions between macrophages and endothelial cells. Top: Macrophages are closely associated with the outer wall of endothelial cell tubules. Bottom: Macrophages bridge adjacent vascular structures. Reprinted with permission from Moore et al. [63]. (C) Macrophages increase vascular germination. Endothelial cells (red); Left: DAY0; Right: DAY5. The images show the increased number and length of endothelial cells at day 5 of co-culture with macrophages. Reprinted with permission from Tattersall et al. [58]. (D) Macrophages and endothelial cells are closely physically associated. Left: White surrounds TNR-positive macrophages; right: F4/80+ macrophages at the distal retinal vessels. Arrowheads indicate anastomosing sprouts or vascular branchpoints. Reprinted with permission from Outtz et al. [61]. (E) Schematic diagram of the method for generating fused vasculature and brain organoids (fVBOr). EB, embryoid body; NE, neuroepithelium; VP, vascular progenitor cells; VO, vascular organoids; BOr, brain organoids. Reprinted with permission from Sun et al. [74]. (F) Encapsulation of endothelial cells and macrophages into PEG-based hydrogels. Cells are mixed with PEG macromolecular monomers (PEG-PQ-PEG and PEG-RGDS). Crosslinking occurs upon exposure to white light, encapsulating cells into PEG-based hydrogels. Reprinted with permission from Moore et al. [65]. (G) (Upper) Infiltrated monocytes release multiple angiogenic cytokines and growth factors (*i.e.*, VEGF) that recruit SMCs and ECs onto scaffolds. ECs and SMCs are properly organized into mature vascular structures on the luminal surface of the scaffold. Upon degradation of the scaffold, monocytes migrate away, leaving behind new blood vessels that are completely autologous. (Lower) Immunohistochemical VEGF staining of stents at weeks 1, 6, and 10 post-implantation shows persistent VEGF expression (brown, positive VEGF expression) throughout TEVG development. (Bottom) Micrographs, 400× magnification. Reprinted with permission from Roh et al. [57].

However, the importance of anti-inflammatory M (IL-4)-like macrophages in muscle regeneration remains controversial, as negative results obtained from IL4ra$^{f/f}$LysM^{cre} mice [48,49] may be qualified by reported limitations in the use of Lyz2-cre mice to dissect the contribution of these cells *in vivo* [50]. Wnt signaling in macrophages may also be a key pathway driving parenchymal regeneration in models of liver injury. Following cell death, macrophages phagocytize hepatocyte debris to induce Wnt3a, which leads to canonical Wnt signaling in nearby hepatic progenitor cells, promoting their specificity toward hepatocytes [51].

4. Macrophages in vascularization of tissue-engineered organ grafts

Tissue engineering generates tissue alternatives to donor materials to repair or replace damaged tissues or organs [52]. Vascularization is critical for the successful grafting of tissue-engineered constructs [53]. Following implantation, engineered tissue requires a network of blood vessels to supply nutrients and oxygen to cells at the transplant site, a complex process that requires the formation of a new, reconstituted host vasculature integrated with the original vascular network. However, vascularization remains a major obstacle to the large-scale clinical application of tissue engineering.

The formation of natural vascular networks involves two processes, vasculogenesis and angiogenesis (Fig. 5A). Vasculogenesis is the differentiation of angioblast cells into endothelial cells, which proliferate to form a primitive vascular network in vascularized tissue, while angiogenesis is the germination of new blood vessels from an original vascular network. Macrophages are important mediators of tissue vascularization that regulate vascular growth in various tissues and play similar roles in tissue-engineered graft vascularization by participating in angiogenesis. Therefore, it is beneficial to incorporate macrophages into biomaterials. Host macrophages have been utilized in several tissue engineering approaches to improve tissue maturation, repair, and regeneration. Understanding macrophage responses during biomaterial design is therefore critical for tissue engineering.

4.1. Macrophages in graft vasculogenesis

Tissue-engineered grafts induce acute and chronic inflammatory reactions following implantation, which influence graft vascularization. Acute inflammatory responses usually resolve within one week, followed by a chronic inflammatory response characterized by the presence of mononuclear cells and lymphocytes [54]. Grafts can only initiate a regenerative pathway once the inflammatory response stabilizes and the surrounding microenvironment conditions are suitable for tissue remodeling [55]. Immunity-associated macrophages are involved in angiogenesis.

4.1.1. Positive effects

Acute inflammatory response following implantation is critical for the initiation of angiogenesis. Following tissue-engineered graft im-

plantation, DAMp-related molecular patterns (dAMPs) are released and recognized, leading to monocyte attachment and activation. Activated monocytes promote M-CSF and MCP1 release and mononuclear macrophage colonization in biomaterials.

During the first week post-implantation, macrophages attached to the implant are transformed to the M1 phenotype under the action of pro-inflammatory cytokines (such as IL-1) and maintain their phenotypes under TLR and TNF-α signals, performing various functions such as participating in leukocyte recruitment and releasing MMPs and reactive oxygen species (ROS) to degrade biological materials. Endothelial cell chemotactic factors can also be released upon increased inflammation. One week later (within a month post-implantation), M1 macrophages adapt the M2 phenotype, which allows for phagocytosis of necrotic tissue under the influence of the signaling cascade of other white blood cells and apoptotic cells. M2 macrophages release anti-inflammatory cytokines, such as IL-10, while producing extracellular stromal components, such as type I and type IV collagen, elastin, fibronectin, and aminoglucan, which promote ECM remodeling essential for late endothelial cell migration and new blood vessel growth.

4.1.2. Side effects

Foreign body reaction (FBR) that occurs after graft implantation can negatively affect the transplant site by inhibiting graft integration and reducing the blood supply. The presence and type of macrophages determine FBR coordination, whereas, in the absence of macrophages, no histological features of FBR exist. Macrophages abound at the implant site within a few days of implantation, attempting to engulf foreign substances and releasing numerous signaling molecules that influence the behavior of other cells and determine long-term FBR outcomes. When the graft biomaterial microstructure cannot support cell infiltration and the material resists degradation *in vivo*, macrophages may mediate the wrapping of the graft with fibrotic tissue, preventing vascularization. Specifically, M1 macrophages and foreign body giant cells release fibroblast inducers, induce fibroblast proliferation, promote collagen deposition, and form a fibrous envelope on the graft surface.

4.2. Macrophages in graft angiogenesis

The construction of a functional vascular network is important for graft–host integration and requires the formation of new and reconstituted host vasculature to integrate with the original vascular network. Excessive inflammatory response caused by M1 macrophages is detrimental to angiogenesis. M2-phenotypic macrophages, including the M2a and M2c subgroups, are associated with anti-inflammatory responses that trigger cell recruitment, differentiation and proliferation, angiogenesis, and biomaterial degradation and absorption, which promote angiogenesis and tissue regeneration. However, data regarding the angiogenic potential of different macrophage phenotypes are conflicting. Indeed, some studies have proposed that M1 macrophages are important initiators of angiogenesis, M1 and M2c macrophages induce endothelial cell germination, and M2a macrophages promote anastomosis. M2f macrophages secrete anti-inflammatory mediators, which are associated with endothelial cell migration and angiogenesis. Other studies have reported conflicting potential causes of temporal differences, with the short-term presence of M1 macrophages enhancing angiogenesis, while the long-term causing vascular regression. Therefore, M1 macrophages may support angiogenesis over time by changing their behaviors [14,33]. M2-like macrophages release arteriogenic factors, leading to smooth muscle cell recruitment and growth, thus promoting angiogenesis and tissue regeneration in a mouse hind limb ischemic model [51]. M2a macrophages also promote angiogenesis in the kidney, whereas M2c macrophages promote fibrosis under given conditions in vascular repair. In mice, subcutaneous injection of Matrigel increases the number of endothelial cells and tubular structures in M2-rich implants. However, VEGF upregulation in M1 macrophages and an increased M1/M2 ratio are also related to vascularization [33]. Therefore, the roles of various macrophage populations in angiogenesis are controversial, and the associated mechanisms remain unclear.

4.2.1. Macrophage factor secretion in angiogenesis

Yolk sac macrophages are involved in vascular development, *e.g.*, through increasing capillary density, promoting vascular recombination and pruning, acting as cell chaperones of endothelial apex cells, and differentiating into pericytes as Sertoli cells. Macrophages contribute to the formation of collateral circulation following arterial occlusion [56] and play a similar role in graft vascularization by triggering the instigating inflammatory mechanisms [57].

4.2.1.1. Positive effects.

Macrophages act on endothelial cells or pericytes by secreting cytokines, as suggested by the results of combining mouse macrophages, human umbilical vein endothelial cells (HUVECs), and human brain vascular pericytes (HBVPs) with carrier microbeads and embedding them in a fibrin gel to mimic the vasculogenic microenvironment. Specifically, the presence of macrophages increased the length and number of germinated HUVECs without evident macrophage migration or spread (Fig. 5C) [58]. Bone marrow-derived macrophages transplanted into the site of segmental nerve injury significantly increased the number of endothelial cells at the site of injury and induced regeneration following injury by promoting angiogenesis [59]. The results of *in vitro* germination tests and *in vivo* experiments on the role of different macrophage subtypes in biological scaffold vascularization showed that M1 macrophages are important initiators of angiogenesis by expressing initiation-associated genes, such as VEGF, FGF2, IL-8, and CCL5/RANTES, along with high levels of heparin-binding EGF-like growth factors (HBEGFs) associated with pericellular recruitment [33]. M1 macrophage-secreted inflammatory cytokines TNF-α and IL1-β drive endothelial cell germination by promoting apical cell phenotypes and stimulating endothelial cell recruitment to support pericytes. M2a macrophages support angiogenesis by recruiting pericytes, regulating M1 macrophage signal transduction, and expressing and secreting high levels of the pericyte-stabilizing PDGF-BB form. Without this effect, VEGF-stimulated blood vessels leak, become immature, and easily fade. M2b macrophages secrete TGFβ1, which facilitates endothelial cell and supporting cell interaction, regulates vascular stability, and promotes endothelial cell migration and angiogenesis. All macrophages secrete high levels of MMP9, an effective stimulator of angiogenesis both *in vitro* and *in vivo* and an important factor in vascular remodeling, contributing to ECM remodeling to allow endothelial cell migration and isolated VEGF release through proteolysis. Thus, M1 macrophages initiate angiogenesis, whereas M2 macrophages promote vascular maturation.

4.2.1.2. Side effects.

Secretion of other cytokines negatively affects angiogenesis. For example, M2a macrophages express high levels of tissue inhibitor of matrix metalloproteinase–3 (TIMP3), which inhibits MMP9 enzyme activity and blocks MMP9–VEGFR2 binding to inhibit VEGF signaling, thus effectively inhibiting angiogenesis. TIMP3 also blocks TNF-α release [60].

4.2.2. Macrophage cell–cell contact in graft angiogenesis

Macrophages can regulate graft angiogenesis through direct cell-cell contact. Macrophages and endothelial cells exhibit a close physical connection. Specifically, macrophages in the retina are localized between Dll4-positive tip cells and vascular branch points, which activates Notch signaling, a key regulator of angiogenesis, vascular differentiation, and vascular integrity (Fig. 5D) [61]. Macrophages in the brain promote VEGF-mediated tip cell induction downstream of tip cell fusion, promoting anastomosis by forming a bridge between adjacent buds, supporting macrophages as essential regulators of tip cell formation and vascular anastomosis [62]. Macrophages can also enhance angiogenesis and increase the number and length of endothelial buds in a macrophage Notch signaling-dependent manner [58]. Similarly,

macrophages interact with two adjacent endothelial cells in a polyethylene glycol (PEG)-based 3D hydrogel to support cell-like bridging endothelial cells (Fig. 5B) [63]. M1 macrophages reduce capillary formation when co-cultured with endothelium, whereas conditioned medium from M1 and M0 macrophages enhance capillary formation, suggesting that direct cell-cell contact between M1 macrophages and endothelial cells underlies vasculogenesis inhibition [64]. Finally, results from co-culturing primary aortic endothelial cells (AECs) and macrophages encapsulated in a bioactive PEG hydrogel, a highly controllable 3D model, showed that the presence of M2 macrophages increased capillary formation three-fold, whereas M1 macrophages inhibited vascular network formation (Fig. 5F) [65].

4.3. Macrophage-mediated vascularization in tissue-engineered grafts

4.3.1. Tissue-engineered vascular grafts (TEVGs)

The goal of TEVGs is to construct a duct-like structure that can remodel and grow following transplantation, respond well to hemodynamics, and maintain patency. For TEVG vascular remodeling, the graft should be an appropriate scaffold to reintroduce and regulate the smooth muscle cell endothelial lining and outer fibrous layer in a controlled manner. Within one week of implantation, inflammatory cells are attracted from the perivascular tissues to the TEVG outer surface. Depending on the scaffold microstructure, monocytes, which will transform into macrophages, and other types of leukocytes promote capillary-rich adventitia generation, thereby providing sufficient cell influx to the graft scaffold and creating conditions for vessel wall regeneration [55]. The role of macrophages in vascular remodeling can be summarized as follows: mononuclear macrophages are recruited to biological TEVG scaffolds after implantation, releasing various angiogenic cytokines and growth factors to recruit smooth muscle cells and endothelial cells to the scaffold.

For example, M2 releases TGFβ and other molecules such as Arg-I and MCP-1, which induce smooth muscle cell migration and proliferation. Upon scaffold degradation, the mononuclear macrophages migrate away, leaving behind a new, entirely autologous vessel of endothelial and smooth muscle cells (Fig. 5G) [57].

The formation of a functional endothelium is essential to control inflammation and the expression of infiltrating cell phenotypes. Macrophages are involved in re-endothelialization. Indeed, M2 macrophages release VEGF, IGF binding protein 3 (IGFBP-3), and stromal cell-derived factor 1 (SDF1) to induce endothelialization, with mature endothelial cells maintaining the M2 state through NO activity and IL-4 release. A proposed model for TEVG endothelium generation by circulating monocytes involves the action of M2e cells. After activation of the cellular Wnt pathway under shear stress, such monocytes with initially spindle-shaped morphology can proliferate and form the compact colony-forming and cobblestone morphology characteristic of endothelial cells, express key endothelial cell-related genes, and produce NO functions [66]. Human peripheral blood cells can also differentiate into endothelial cells and form colonies similar to mature vascular endothelial cells [67].

4.3.2. Tissue-engineered bone

The bone tissue is highly vascularized. Cortical bone is permeated by a network of vascular channels, and the interior of the marrow cavity is highly vascularized. Bone tissue can self-heal; however, severe tissue defects can lead to poor bone formation and non-union. Biomaterial-based tissue engineering is an important therapeutic approach. The regeneration of large defects in bone requires rapid vascularization to provide oxygen and nutrients, although when bone replacement materials are implanted into large bone defects, insufficient vascularization often leads to poor regeneration. Achieving early vascular formation in large-area bone transplantation remains challenging [68].

Biomaterial bone scaffolds represent ECMs synthesized from multiple biological materials, such as degradable multimers or ceramics. The resulting 3D matrix supports the attachment, recruitment, growth, proliferation, and differentiation of osteoblasts and induces or supports blood vessel formation. Inflammatory cell recruitment is important for biomaterial-induced bone remodeling. Upon biomaterial implantation into a bone defect, the tissue response included infiltration of M1 and M2 macrophages into the implantation site within 2 months. In turn, at 14-day follow-up after transplantation, macrophages had adhered to the scaffold surface and contributed to biomaterial degradation. The production of VEGF at the graft site provides an angiogenic signal and strongly promotes vascularization, and the depth of stent vascularization is related to the depth of macrophage infiltration into the stent interior [69].

Scaffold composition and degradation rate may serve to regulate macrophage function to achieve vascularization. For example, materials designed to release Na, Ca, Si, and P ions during macrophage-mediated degradation can induce angiogenesis [70]. Furthermore, evidence suggests that the macrophage polarization state, which is important for the revascularization of tissue-engineered bones, is highly sensitive to the physicochemical properties of biomaterials. Advances in biomaterials research have allowed for good control of surface properties to exploit this feature. For example, the sequential activation of macrophages from M1 to M2 can promote the vascularization of scaffolds used in bone regeneration.

4.3.3. Tissue-engineered muscle

Skeletal muscle has the unique ability to fully recover its structure and function after minor acute injury. However, repairing large muscle defects is a major challenge in regenerative medicine and requires the application of tissue engineering strategies. Biomaterials should be biocompatible to minimize local host immune responses. However, active modulation of immune cells through biomaterials can also effectively control immune responses and promote muscle tissue regeneration and biomaterial integration, with macrophages in particular being important for the regeneration and repair of damaged musculoskeletal tissue [71].

Muscle regenerative ability is attributed to permanent resident stem cells called muscle satellite cells (MuSCs). Since incorporating macrophages into tissue-engineered muscles may enhance muscle regeneration, the incorporation of resident bone marrow-derived macrophages into human-derived 3D muscle cell cultures may support satellite cell proliferation and differentiation. *In vivo*, the macrophages in the implanted engineered muscle could enhance blood vessel ingrowth and form endogenous blood vessels with normal blood perfusion [72].

4.3.4. Vascularized organoids

The main cause of growth arrest or cell death in tissue-engineered organoids is the lack of adequate oxygen and nutrient supply; moreover, organoid maturation is affected by nutrient supply limitations. Therefore, the construction of organoids with functional vascular networks is necessary for most highly metabolic organs such as the heart, liver, kidney, and brain.

Brain organoids have been used to model brain development and related diseases. Microglia are macrophages located in the brain and spinal cord that provide the immune defense of the central nervous system (CNS). Functional neuronal and vascular networks in brain organoid grafts included microglia that had integrated into multiple regions of the host brain, and large numbers of host-derived microglia that had migrated into the whole graft, which consequently exhibited typical branching morphology [73]. Vascularized brain organoids have been obtained through the fusion of blood vessels with vascular and brain organoids induced and cultured *in vitro* (Fig. 5E). Culture of mature vessel-like organs in a medium containing neurotrophic factors allowed vascular organoids to acquire cerebrovascular characteristics and induced numerous microglia and vascular cells. Notably, the microglia

were active in response to the immune stimulation of the fused brain organoids and exhibited phagocytic capacity [74].

Recruitment or addition of macrophages is also a means of vascularization in other types of tissue-engineered grafts. For example, Kupffer cells, which exist in the hepatic sinusoids as macrophages in the hepatic sinus, have been incorporated into the bio-ink design in 3D-printed liver [75]. Myocardial revascularization, or neovascularization, is an important therapeutic target for alleviating tissue ischemia caused by vascular diseases and restoring cardiac function. In myocardial tissue, cardiac resident macrophages ensure steady-state cardiac function through phagocytosis, cellular phagocytosis, exon elimination, and the promotion of electrical conductivity [76]. Overexpression of the chemokine MCP-1 in the heart leads to macrophage infiltration, matrix metalloenzyme secretion, and the formation of tunnels containing red blood cells in the myocardium to provide a basis for revascularization.

5. Biomaterial regulation of macrophages promotes vascularization of injured tissues and following organ transplantation

Immunomodulatory biomaterials can harness the inherent regenerative power of endogenous cells and stem cells recruited by activated immune cells from their niche to distant sites [77], to facilitate regeneration and tissue remodeling. Stem cell recruitment, also known as stem cell homing [78], is an innovative strategy in regenerative medicine to enhance the regenerative power of treatment options. Following recruitment, the microenvironment provided by biomaterial scaffolds appropriately guides stem cell proliferation and differentiation into different cell types, promoting tissue regeneration. In addition, biomaterial composition and structure can induce macrophage polarization to further support endogenous stem/progenitor cell homing, differentiation, and tissue regeneration [78].

5.1. Biomaterials design schemes on promoting macrophage infiltration into tissue-engineered organs in vivo

The success of graft vascularization is related to the number and type of infiltrating cells. A graft microstructure not capable of supporting macrophage infiltration will lead to vascularization failure. Macrophage infiltration promotes angiogenesis and reduces fibrosis and scar formation. Thus, the ability of the implant to promote cellular infiltration and inward vascular growth is key to modulating acute and chronic inflammation, promoting tissue remodeling, angiogenesis, and regeneration, and achieving durable soft-tissue repair.

5.1.1. Physical structures that support macrophage infiltration

In natural tissues, cells tend to reside in porous ECMs, which provide geometrically restricted space and facilitate nutrient trapping, waste, and diffusion of intercellular communication between cells. In implants, macrophage infiltration is related to biomaterial porosity and pore size. Compared with non-porous implants, porous materials implanted in soft tissue modulate macrophages to promote angiogenesis and reduce fibrosis and scar formation [79].

A potent angiogenic response, accompanied by M2 macrophage infiltration, could be mediated by a material pore size of 30–40 μm, as compared with the results from non-porous or 160 μm porous implants (Fig. 6A) [80]. Additionally, a bio-scaffold allowing for engineering 3D *in vitro* tissue models, wound dressings, or *in vivo* regenerative tissues has been developed using a gas-foaming technique to obtain a 3D scaffold that promotes macrophage infiltration into the interstices between nanofiber layers; moreover, microporous arrays applied on the scaffold under cryogenic conditions could further enhance macrophage infiltration. Furthermore, whereas macrophages remained only on the material surface after implantation of a conventional nanofiber membrane, the developed scaffold supported macrophage infiltration from the material surface via micropores, and subsequently significantly increased neovascularization (Fig. 6C) [81]. An injectable biomaterial for injury repair has also been reported to simulate the ECM and mechanical properties of soft tissue. Specifically, by bonding electrostatically spun polycaprolactone fibers and a hyaluronic acid hydrogel network (PCL-nanofiber-HA hydrogel composite), a porous structure that supports cell migration and organization *in vitro*, a 3D vascular network was formed that allowed for host macrophage infiltration and angiogenesis following implantation *in vivo* [82].

5.1.2. Chemical factors that induce immune cell infiltration

Biomaterial design has focused on the delivery of small molecules, proteins, and cells to facilitate cell infiltration, degradation, vascularization, or innervation of the scaffold. Additionally, the host response to biomaterials is strongly influenced by chemical factors. Chemical signals influence a variety of cellular events such as adhesion, migration, proliferation, and differentiation. Natural ECM can provide chemical signals to cells and regulate cell behavior.

Multidomain peptide (MDP) hydrogels are a class of nanofiber materials with promising biomedical applications and the ability to incorporate various chemical functionalities into the nanofiber scaffolds. Whereas both a nanofiber peptide hydrogel without small-molecule drugs, cells, or proteins, and a lysine-based single-component MDP hydrogel K2 (SL) 6K2, which stimulated inflammation following implantation, supported rapid cellular infiltration following the post-implantation immune response, the modified hydrogel also formed a dense mature vascular network (Fig. 6E) [83]. In turn, a hydroxyproline-based self-assembled nanofiber peptide hydrogel caused infiltration of F4/80+ macrophages with complete graft infiltration over time after implantation in mice, but the inflammatory response was weaker than that from the lysine hydrogel and vascularization did not occur. Comparison of the early inflammatory host response of four MDP hydrogels, K2 (SL) 6K2, R2 (SL) 6R2, and E2 (SL6E2 and D2 (SL6D2) in a subcutaneous injection model, based on lysine arginine, glutamate, and aspartate, showed that positively charged lysine and arginine hydrogels induced a high degree of macrophage infiltration and angiogenesis, whereas negatively charged glutamic acid and aspartic acid-induced weak macrophage infiltration and no angiogenesis in the graft (Fig. 6B) [84]. Lopez-Silva et al. reported that Zn-based biomaterials affect angiogenesis in bone repair and observed an increase in vascular endothelial cells around the Zn implant and enhanced angiogenesis with distance-dependent characteristics. In a specific concentration range, the authors also showed that Zn promoted the polarization of M0-type macrophages to M2-type while inhibiting M1-type polarization [85].

5.1.3. Biological means of inducing macrophage recruitment

The recruitment and retention of macrophages by soluble chemokines in the implantation niche are key determinants of microvascular growth and remodeling, and ultimately of implant success. For example, the delivery of VEGF-165 and PDGF-BB from one-to-one polymer scaffolds engineered with different mechanical multi-angiogenesis factors resulted in the rapid formation of mature blood vessels (Fig. 6F) [86].

Heparin-derived SDF-1 α in a PEG-diacrylate hydrogel could increase angiogenesis in hydrogels and microvascular growth upon implantation in mice by preferential recruitment of anti-inflammatory monocytes expressing the SDF-1 α receptor, CXCR4. (Fig. 6D) [87]. Implantation of a PEG-diacrylate hydrogel containing PDGF-BB/FGF2 into corneal microcapsule model mice activated macrophage infiltration and migration toward the PDGF-BB/FGF2 hydrogel, suggesting that macrophages are associated with neovascularization, with a role in endothelial tip cell migration, anastomosis, and pericyte-like behavior (Fig. 6G) [88]. Moreover, hydrogels that simultaneously released VEGF and M-CSF1 produced a robust and stable angiogenic response comparable to that induced by PDGF-BB/FGF2, demonstrating that M-CSF1-mediated macrophage recruitment is sufficient to improve the angiogenic response to VEGF, although M-CSF1 alone increased

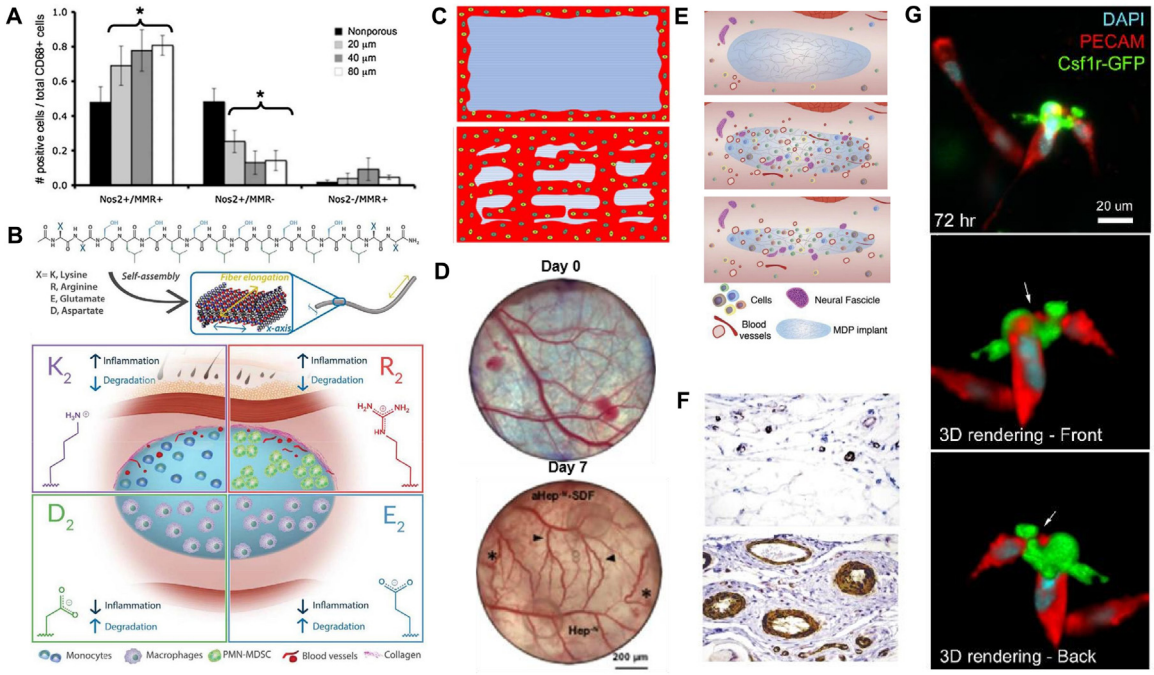

Fig. 6. Physical structures that support macrophage infiltration. (A) Identification of MΦ, M1, and M2 phenotypes using CD68+ staining according to NOS2 and MMR status. M1 and M2 phenotypes were determined using NOS2 and MMR ($n = 4$; $P < 0.05$); NOS2+/MMR+MΦ cells increased significantly at all porous implant sites. Compared with the non-porous structure, NOS2−/MMR+MΦ cells showed an increasing trend in the 40 μm porous structure. ($P = 0.06$) Reprinted with permission from Madden et al. [80]. (B) The chemical MDP affects the host's immune response to hydrogels. Positively charged lysine and arginine hydrogels can induce high infiltration of macrophages; negatively charged glutamic acid and aspartic acid induce weak macrophage infiltration. Reprinted with permission from Lopez-Silva et al. [84]. (C) Top: Traditional nanomembranes; bottom: expanded and perforated nanofiber scaffolds. Distribution of M1 (yellow) and M2 (blue) macrophages is shown. Reprinted with permission from Jiang et al. [81]. (D) Dorsal skinfold tissue treated with aHep-N-SDF, showing vascular increase on the seventh day. Vascular caliber (arrow) and tortuosity (asterisk) are shown. Reprinted with permission from Krieger et al. [87]. (E) Diagram showing the time course of hydrogel remodeling, starting with cell infiltration (top), followed by vascularization and innervation, and slow degradation (bottom). Reprinted with permission from Moore et al. [83]. (F) Dual delivery of VEGF and PDGF induces vascular wall cells, as shown using α-smooth muscle actin staining. Top: untreated stent; bottom: VEGF and PDGF double-release stents. Reprinted with permission from Richardson et al. [86]. (G) Examples of physical binding of macrophages to HUVECs, and 3D renderings of a z-stack at different angles, The images show that macrophages bridge the connection between two independent structures. Reprinted with permission from Poché et al. [88].

macrophage recruitment to the cornea but failed to elicit an angiogenic response [89]. Implantation of a bifunctional hydrogel with sustained release of the sphingosine analog FTY720 and SDF-1α using heparin derivatives into mouse skin led to the co-release of FTY720 and SDF-1α and bone marrow cell recruitment to the implant interface, as pro-regenerative monocytes have high expression of the bioactive lipid receptor sphingosine-1-phosphate receptor 3 (S1PR3) and CXCR4. Although the *in vivo* delivery of FTY720 or SDF-1α facilitated Ly-6C^{low} anti-inflammatory monocyte recruitment, co-delivery enhanced CD206+ macrophage recruitment and accumulation in the tissue surrounding the gel, facilitating revascularization in the implant vicinity (Table 4) [90].

5.2. Biomaterials design schemes on regulating macrophages to favor vascularization of tissue-engineered organs

5.2.1. Controlled-release strategy to modulate the balance between M1 and M2 phenotypes

M2 macrophages recruited by IL-4 determine fibrous capsule formation *in vivo* post-implantation [91], whereas persistent M1 macrophages in the absence of M2 macrophages lead to chronic inflammation and delayed healing. Nevertheless, both M1 and M2 macrophages are required for scaffold vascularization, and vascularization and integration cannot be achieved if macrophage phenotypes are overly skewed in the M1 or M2 direction [33]. Therefore, the use of grafts to adjust the balance between M1 and M2 phenotypes is essential for vascularization and graft–host integration. Acellular bone scaffolds for sequential cytokine release, including IFN-γ physically absorbed onto the scaffolds and IL-4 bound by biotin-streptavidin, showed rapid release of physically adsorbed factors but continuous and slow release of biotin-streptomycin-bound factors (Fig. 7A, 7B). The pro-inflammatory cytokine IFN-γ was released first to enhance the natural M1 response to injury, followed by the anti-inflammatory cytokine IL-4 to promote the M2 phenotype, which enhanced stent vascularization after subcutaneous implantation in mice [92]. For treating bone defects, IFN-γ was loaded onto a 5% calcium silicate/β-tricalcium phosphate (CaSiO3-β-TCP) biological scaffold. After scaffold implantation, IFN-γ release stimulated macrophage M1 polarization at an early stage, followed by Si release, which induced macrophage M2 polarization upon scaffold degradation. Compared with the control group, IFN-γ@$CaSiO_3$-β-TCP scaffolds formed more blood vessels *in vitro* and *in vivo* [93]. Biphasic cytokine release of MCP-1 and IL-4 from a biomimetic MDP hydrogel that sequesters cytokines in a nanofibrous matrix resulted in spatiotemporal activation of monocytes and macrophages, creating a pro-angiogenic environment [94]. A double hydrogel layer system on titanium dioxide nanotubes (tNTs) as a reservoir to regulate the release of IL-4 and IFN-γ, in which IL-4 was loaded into the nanotubes and IFN-γ was located between the hydrogels (Fig. 7C), could regulate the macrophage M1 to M2 phenotype switch through the sequential action of the two cytokines to promote angiogenesis [95]. Zhang et al. found that reduced lactate secretion in endothelial cells with glycolytic regulator pfkfb3 deficiency reduced M2 macrophage differentiation that promotes angiogenesis, thereby impair-

Table 4
Comparison of various hydrogels biologics, from factors, release mode, *in vivo* and *in vitro* effects.

Materials	Biological factor	Mode of release	*In vitro*	*In vivo*
PEG-DA hydrogel	SDF-1α	Heparin combined with sustained release	Mediated the recruitment of monocytes, participated in the growth of microvascular network, and sent signals by binding to CXCR4 on the cell surface.	Recruitment of anti-inflammatory mononuclear cells, selective recruitment along the peri-implant arterioles, promotion of the growth and maturation of the microvascular network.
PEG-DA hydrogel	PDGFBB/fGF2		As a chemokine for macrophages, it acts in a pro-vascularization manner, supporting vascularization and arteriogenesis	Role of macrophages in endothelial tip cell migration and junction and pericell-like behavior.
Difunctional PEG-DA hydrogel	FTY720 and SDF-1α	Heparin derivative (Hep-N) and albumin	Bioactive lipid receptor 1-phosphosphingosine receptor 3(S1PR3) and matrix derived factor-1α (SDF-1α) receptor CXCR4 were highly expressed in proregenerative monocytes	The co-release of FTY720 and SDF-1α resulted in superior recruitment of bone marrow cells to the implant interface, enhanced the early accumulation and persistence of $CD206^+$ macrophages in differentiated wage-healing tissues around the gel, and promoted the co-dilation of vasculature near the implant.

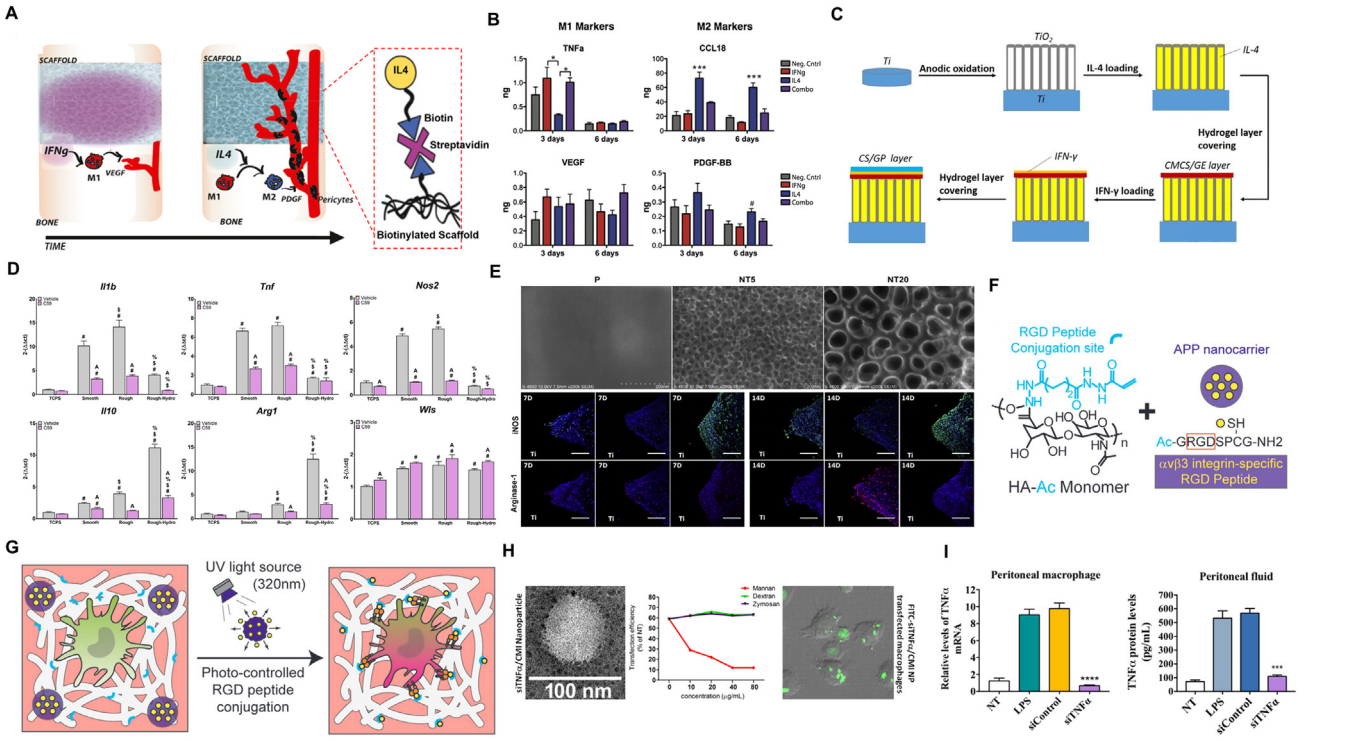

Fig. 7. Physicochemical properties of materials that regulate macrophage polarization. (A, B) A scaffold with physical adsorption of IFN-γ promoted the phenotypic transformation of macrophages. IFN-γ was physically loaded on a decellularized bone scaffold, and IL-4 was attached to the scaffold via biotin-streptavidin binding to achieve sequential release. M1 macrophages release the angiogenic growth factor VEGF, which recruits endothelial cells and initiates the angiogenic process. Subsequent release of IL-4 by the scaffold converts M1 macrophages to an M2 phenotype. M2 macrophages secrete factors, such as PDGF-BB, that recruit pericytes to stabilize the growing vasculature; B cytokine secretion by macrophages seeded on scaffolds. $^*P < 0.05$ using one-way ANOVA and Tukey's post-hoc analysis; $\#P < 0.01$ and $^{***}P < 0.001$ using one-way ANOVA followed by Dunnett's post-hoc analysis (mean +/- SEM, $n = 5$). Reprinted with permission from Spiller et al. [92] (C) Schematic representation of titanium dioxide nanotube (TNT) preparation, cytokine loading, and hydrogel layer coverage. Reprinted with permission from Chen et al. [95] (D) Expression of inflammatory genes in primary murine macrophages and macrophages treated with C59 (Wnt inhibitor) cultured on different properties titanium surfaces. Reprinted with permission from Abaricia et al. [97]. (E) Nanostructured titanium surface characterization and macrophage polarization moderated by its implantation *in vivo*; M1 polarization (iNOS, green) and M2 polarization (ARG, red). The NT20 surface significantly induced M1 polarization, whereas the NT5 surface promoted M2 polarization. Scanning electron microscope: Scale bar, 50 nm; immunofluorescence: Scale bar, 100 μm. Reprinted with permission from Ma et al. [101]. (F, G) Thermoresponsive HA hydrogel nanocomposites with photocontrolled 3D ECM adhesion sites for dynamic macrophage immunomodulation. Reprinted with permission from Wang et al. [107]. (F) Generation process of photoresponsive HA hydrogel nanocomposites. (G) Under UV irradiation, RGD peptides covalently bind (blue "Y") to cross-linked HA hydrogels (white), activate $\alpha v\beta 3$ integrin macrophage expression in dynamic 3D HA-ECM composites, and polarize macrophages. (H–I) Mannose-functionalized Curdlan nanoparticles (CMI) mediate short-interfering RNA (siRNA) targeted delivery to macrophages. Reprinted with permission from Ganbold et al. [114]. (H) Transmission electron microscope images showing CMI/siRNA spherical nanoparticles with diameters between 50 and 80 nm. CMI/FITC-siRNA was transfected into primary macrophages. (I) Intraperitoneal injection of a CMI/siTNFα complex induced a significant downregulation of LPS-stimulated TNFα expression in mouse peritoneal macrophages.

Table 5
Biomaterials stimulation of macrophages of different phenotypes.

Materials	The factors that stimulate M1 and their release mode		The factors that stimulate M2 and their release mode	
Acellular bone scaffold	INFg	Physical absorption	Il-4	Biotin-streptomycin affinity binding
CaSiO3-β-TCP scaffold	IFN-γ	Physical absorption	Si	Macrophages degrade the material and release it
Biphasically released biopolypeptide domain hydrogels	MCP-1	The nanofiber matrix isolates two factors		Il-4
Titania nanotube dual hydrogel layer system storage (tNT)	IFN-γ	Located between two hydrogel layers	Il-4	Loaded in tNT
Tio2nanotubes (TTNTs)	IFN-γ	Hydrogel coating	Il-4	Il-4 is loaded into Tio2 nanotubes (TTNTs)
Bioactive glass composite scaffold	IFN-γ		$Sr2^+$	$Sr2^+$ in SrBG

ing ischemic hindlimb revascularization and muscle regeneration. The addition of lactic acid to pfkfb3-deficient EC restored M2-like polarization and improved muscle reperfusion and regeneration (Table 5) [96].

5.2.2. Modulation of macrophage polarization using the physicochemical properties of biomaterials

5.2.2.1. Influence of physical properties of the material surface. The hydrophilic surface of a material regulates macrophages by altering their binding to the material. The hydrophilic and hydrophobic properties of biomaterials play a vital role in activating macrophages, with hydrophobic implant-stimulated macrophages producing higher levels of pro-inflammatory proteins and hydrophilic implants producing higher amounts of anti-inflammatory proteins (Fig. 7D) [97]. The physical parameters of the material also regulate macrophage behavior and phenotype. Substrate hardness is frequently studied as it affects various cellular and tissue functions [98]. Engineered 3D fibrous scaffold materials based on natural biopolymers influence macrophage polarization, with the M2 phenotype expressed on a harder matrix, whereas glycosaminoglycans (GAGs) inhibit this polarization effect [97]. Biomaterial surface roughness can regulate macrophage polarization by altering cell adhesion, diffusion, extension, and movement [99]. In particular, the use of surface topography to elongate macrophages promotes a pro-regenerative M2 phenotype. Nanosurfaces on the peptide implant surface can regulate the macrophage inflammatory response and improve implant performance (Fig. 7E). Anodization of titanium material at 5 and 20 V, expressed as NT5 and NT20 titanium grafts, respectively, formed a multitubular nanostructured surface, with the roughness of the prepared Ti surface increasing as the anodizing voltage increased. After material implantation, M1 polarization on the NT20 surface was enhanced, and considerable iNOS was induced around the implant. The M2 ratio on the NT5 surface was significantly enhanced, which induced Arg-1 production and inhibited iNOS synthesis. Thus, modulating grafts by manipulating the biomaterial surface nanomorphology to control macrophage polarization states is a feasible strategy [100].

5.2.2.2. Chemical modification of the material surface. Inflammation can be regulated by phagocytosed nanomaterials such as liposomes, polymer particles, and inorganic materials. In MI treatment, the released mesoporous silica nanoparticle (MSN) complex significantly inhibits the inflammatory response and promotes local neovascularization by inhibiting M1 macrophage polarization in the infarcted myocardium [101]. In addition, the effect of material surface chemistry on macrophage polarization *in vivo* has been studied using nanoparticles modified with different functional groups (*e.g.*, aldehydes, ketones, nitro groups, or ether). CD40/F4–80 staining revealed more M1 macrophages in the sulfonic acid, nitro, and unmodified nanoparticle implantation sites than in the aldehyde and ketone functional group-modified materials; M2 macrophages did not differ significantly among the groups [102].

Among two biological scaffolds from porcine small intestinal submucosa (SIS), the decellularized autologous SIS scaffold recruited mainly M2 macrophages and displayed structural remodeling at the site of implantation, whereas the carbodiimide-crosslinked SIS recruited mainly M1 macrophages and displayed chronic inflammation [103]. In comparison, the macrophage response induced by knitted polypropylene mesh is mainly M1, whereas M2 is dominant after coating with ECM, which increases the ratio of M2/M1 in the graft and reduces foreign body reaction and fibrosis [104].

5.2.2.3. Properties of materials inside the support structure. Biomaterials that mimic the ECM physical structure or chemical composition can modulate the macrophage phenotype, reduce inflammation, and promote wound healing. Additionally, implants that mimic the natural ECM may facilitate more effective wound integration and the generation of new physiologically compliant tissues [105]. A bioactive polypeptide hydrogel (QK-SF) composed of silk fibroin (SF) and the VEGF-mimicking peptide KLTWQELYQLKYKGI (QK), which regulates macrophage polarization and promotes wound healing, triggered macrophage polarization from M1 to M2, and promoted keratinocyte differentiation and collagen deposition in mouse skin wound models. Upon light-controlled 3D ECM-RGD peptide coupling, a biomimetic photoresponsive HA hydrogel nanocomposite with tunable 3D ECM adhesion sites for dynamic macrophage immunomodulation activated $\alpha v\beta 3$ integrins in macrophages, activated $\alpha v\beta 3$ integrins by periodic UV irradiation, enhanced Arg-1 expression, inhibit iNOS expression, and promoted the transition of M0 to M2 macrophages Similarly, expression of the pro-inflammatory cytokines TNF-α and IL-6 was decreased, whereas anti-inflammatory cytokine TGF-β1 and IL-10 expression was significantly increased (Fig. 7F, 7G) [106].

As a component of the ECM, GAG directly controls cellular function through receptor binding and signal transduction, or by regulating the availability and biological activity of growth factors and cytokines. The GAG HA generally has a strong anti-inflammatory effect, although small, degraded fragments of HA favor the progression of inflammation. A biomimetic ECM based on a non-sulfate GAG HA/collagen (HA/coll) hydrogel regulated the pre-immunomodulatory activity of immune cells, including macrophages, via direct receptor-mediated interactions with cells [107]. The use of micrographic methods to directly control the shape of macrophages and, thus, the macrophage phenotype *in vitro* resulted in M2 cells exhibiting an elongated shape compared to that of M1 cells. Moreover, cell culture media engineered to directly control the shape of cells demonstrated that cell elongation induced polarization to the M2 phenotype, enhanced the effect of M2-induced cytokines, and inhibited the action of M1-induced cytokines [108].

5.2.3. Strategies to regulate macrophage polarization based on gene transfection

MicroRNAs (miRNAs) are an alternative approach to regulating macrophage polarization. Targeted delivery of biodegradable polylactic-co-glycolic acid (PLGA) polymer nanoparticles (NPs) coated with miR-132 to HUVECs increased microvessel formation in the graft [103]. Using acid-degradable PEG-poly (amino ketal) (PEG-PAK)-based micelles, the gene encoding SDF-1α was introduced into human adipose-derived stem cells (hADSCs), improving angiogenesis at the implantation site.

In a transplanted HUVEC gel model of neovascularization, miR-132 enhanced angiogenesis by promoting Ras activation and increasing endothelial cell responsiveness to various growth factors [109]. However, targeted delivery to macrophages remains challenging. As primary mature macrophages are telophase cells that do not divide, most delivery techniques that rely on integrating dividing cells do not work on these cells. Macrophages also have numerous effective degradation enzymes, which can destroy the integrity of nucleic acids and render gene transfection inefficient. Therefore, macrophage-targeting systems must promote active uptake and protect the integrity and stability of nucleic acids.

Sustained miRNA delivery can improve implant-host integration by modulating macrophage phenotypes. miRNA delivery using electrospun poly (caprolactone-ethyl phosphate) nanofiber scaffolds induced macrophage polarization. Histological analysis after implantation revealed the highest density of new vessels in miR-124-treated stents. Alternatively, miR-146a, miR-21, and miR-125 inhibited macrophage polarization toward pro-inflammatory M1 phenotypes in implant settings [110]. Another siRNA delivery system is based on a dextran nanoparticle carrier, where dextran is recognized by macrophages using a pathogen-associated molecular pattern protein (pAMP) and actively internalized by macrophages through receptor-induced interactions [111]. Alternatively, a micellar nanodrug could load M2 macrophage-targeting peptides into pH-responsive PEG to achieve M2–M1 repolarization [112]. A siRNA vector targeting M2 macrophages comprised chemically conjugated mannose, a macrophage-targeting ligand, with the nucleic acid carrier 6-amino-6-deoxygel polysaccharide, and combined the conjugate with siRNA to form nanoparticles that could deliver siRNA to mouse abdominal macrophages (Fig. 7H, 7I) [113]. Finally, as a safe and efficient system to deliver siRNA to macrophages that are difficult to transfect, TNF-α siRNA can be delivered to lipid-activated macrophages (M1) using the transfection agent lipoid 5(L5) and lipoid-polymer hybrid nanoparticles (LPNs) composed of the biodegradable polymer poly (D, L-lactide-hydroxy-ethylate) [114].

5.2.4. Nanodrug delivery strategies based on inflammation and angiogenesis

Nanotechnology has attracted research attention due to its potential application in disease diagnosis and treatment, including inflammation and angiogenesis. Inflammation and angiogenesis drive the onset and progression of various diseases, such as atherosclerosis, cancer, and rheumatoid arthritis, which share several common pathophysiological features, among which monocyte recruitment, macrophage polarization, and enhanced vascular permeability play key roles [115]. Nanomedicine can be designed to address the different features of maladaptive inflammation and angiogenesis for therapeutic and diagnostic purposes. Nanomaterials are highly tunable and can be designed with various characteristics, including size, shape, and surface chemistry, to modulate *in vivo* behaviors of nanoparticles, such as recycling kinetics, cellular uptake, and tissue penetration. In addition, nanoparticles can be used as carriers of different therapeutic cargo, such as small molecule drugs (hydrophilic and hydrophobic), peptides, and nucleic acids. As diagnostic probes, nanoparticles are also highly adaptable and can be labeled for optical, magnetic resonance imaging (MRI), computed tomography (CT), and nuclear imaging methods [116–119].

Considering the high phagocytic activity of monocytes and macrophages, nanoparticles are suitable for dynamic imaging of inflammatory cells in diseases [115]. Infiltrating monocytes and lesion-associated macrophages affect other cells in the microenvironment and induce pathological angiogenesis and local tissue remodeling. The characteristics and structural abnormalities of tumor blood vessels influence the use and delivery capability of nanoparticle drugs. The abnormal structure and high leakage of blood vessels interact with the tumor microenvironment to form an enhanced permeability effect (EPR), and nanoparticles can effectively deliver drugs to tumor tissues through the EPR effect [120].

The application of nanomedicine to target and control the process of angiogenesis is an attractive approach that provides therapeutic and imaging agents. The formation of new blood vessels is under complex control of angiogenic factors, including VEGF and VEGFR.

Inhibition of angiogenesis using polymeric nanoparticles loaded with angiogenesis inhibitors, such as the fumarillin analog TNP-470, has been shown to 1) inhibit tumor growth in mouse models of melanoma, Lewis lung cancer, and ovarian cancer; 2) reduce plaque angiogenesis and advance atherosclerosis in Apoe-/- mice; and 3) inhibit arthritis and prevent bone destruction in mice [115]. Overexpression of the $\alpha v\beta 3$ integrin adhesion receptor by neovasms and attachment of ligands such as antibodies, nanobodies, and peptides to nanoparticles allow for better distribution in the diseased vascular bed and increase their internalization by target endothelial cells [121–123]. In addition, $\alpha v\beta 3$ integrated targeting ligands and nanoparticles have been used for angiogenesis imaging in cancer, atherosclerosis, and arthritis, such as ultrasound, MRI, PET, and SPECT [124–126].

6. Conclusion and perspective

Our understanding of the biology of macrophages in homeostasis and disease has improved over the past several years. The rapid formation of a highly organized vascular network is a prerequisite for the successful repair or regeneration of injured tissues and tissue-engineered grafts. The recruitment of macrophages to the injury site triggers a series of events, such as macrophage secretion of pro-angiogenic growth factors, which improve the vascularization of injured tissues and provision of the oxygen supply required for wound healing. Macrophages thus not only play an important role in the repair and regeneration of damaged tissues but constitute also therapeutic targets for the treatment of injuries. However, understanding and harnessing the heterogeneity of macrophages *in vivo* remains challenging, as macrophages can differentiate into phenotypically and functionally distinct subpopulations depending on organ type, local cytokine milieu, and interactions with other immune cells. Pro-and anti-inflammatory macrophages are the two most frequently studied phenotypes in wound healing repair, fibrosis, and tissue regeneration studies; the extreme heterogeneity of macrophages within tissues both in homeostasis and during inflammation suggests that macrophages cannot be classified simply as “M1” or “M2”.

In this review, we demonstrated that macrophages of different origins in tissues exhibit different phenotypes or functions, such as several tissue-resident macrophages and peripheral blood monocytes, which are recruited and activated through different mechanisms and possess many functional features critical for vascularization during tissue injury and graft remodeling. Macrophages derived from hematopoietic stem cells or yolk sacs have both inflammatory and anti-inflammatory effects and are characterized by the expression of surface markers. Macrophages promote the vascularization of injured tissues through signaling pathways, secretion of related factors, and differentiation into various subsets.

Multiple studies have elucidated the role of macrophages in different tissues, including the heart, peripheral nerves, and bone regeneration. However, since injury is a trigger for both regeneration and inflammatory immune responses, future studies should investigate how macrophage function can be modulated so that regeneration outperforms fibrotic repair.

Macrophages also play a role in the vascularization of tissue-engineered organ grafts including TEVGs and vascularized organoids. Macrophage-mediated mobilization of first-line defenses, particularly phagocytosis and the release of acute inflammatory mediators, is important during this process, but macrophages also mount adaptive alloimmune responses to the graft through antigen processing and presentation, along with providing co-stimulation. The functional diversity of macrophages in organ transplantations is consistent with their heterogeneity. Despite advances in our understanding of the deleterious or

beneficial effects of macrophages in transplantation, the precise mechanisms controlling macrophage function remain poorly understood. Studies employing genomics, transcriptomics, and proteomics techniques will greatly facilitate the definition of human macrophage heterogeneity and diversity and enable the development of selective strategies targeting graft-infiltrating macrophages to improve outcomes for transplant recipients.

Moreover, biomaterials can interact with macrophages to promote graft vascularization. Modern biomaterials continue to advance and develop, opening unlimited potential for customized macrophage-centered therapies, as they can provide a controlled and adjustable microenvironment for macrophages, combining physical structure and material surface chemistry factors, such as size, geometry, surface topography, roughness, hydrophobicity, and the temporal and spatial manifestations of materials to induce immune cells, support macrophage infiltration and polarization adjustment, and regulate macrophages to promote tissue vascularization. Therefore, by manipulating the biomaterial parameters, its potential to elicit a wide variety of responses in macrophages based on its intrinsic properties can be fine-tuned, as these responses are critical determinants of biocompatibility, controlling FBRs and/or tissue integration together with anti-inflammatory responses, and can be used to control the overall fate of macrophages *in vivo*. Although extensive research on novel biomaterials and material design for immune regulation has been conducted in recent years, a renewed focus on directing macrophage behavior to achieve an appropriate immune response must be emphasized. The optimal biomaterial design should act in concert with relevant factors, immune molecules, or cells to induce macrophages to develop a pro-angiogenic phenotype [127]. In the future, both the surface modification of biomaterials and tissue engineering approaches using modified native tissues should be aimed at reducing the potentially damaging effects of inflammation and promoting angiogenesis [128].

The material composition, scaffold physical properties, and other factors must be carefully considered to ensure that the material design achieves the designated goal of regulating the desired macrophage phenotype. A deeper understanding of the physical regulation of macrophages by biomaterials can help to predict biomaterial-host interactions, understand inflammation-related implant failure, and create new strategies for activating and harnessing the power of macrophages for tissue repair, immunotherapy, or drug delivery. In addition, how changing environmental stimuli relate to immune properties in and around the scaffold remains to be determined.

In conclusion, tissue engineering and regenerative medicine facilitate treatment for damaged tissues and end-stage organ failure. The immune response and interaction of neovascularization with biomaterials remain a research hot spot. Better control of the trafficking of immune cells and their local environment is key to the design of biomaterials for this growing field. The development of potential strategies for macrophage response to guide the vascularization of biomaterials is underway.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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