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## Beyond first responders: neutrophils shape biomaterial-guided regeneration

Márcia Monteiro Pereira,<sup>c</sup> Ana Beatriz Sousa<sup>a,b,c</sup> and Judite Novais Barbosa  <sup>a,b,c</sup>

Neutrophils have long been regarded as short-lived effector cells responsible for clearing pathogens and inducing acute inflammation. However, emerging evidence has reshaped this paradigm, revealing remarkable neutrophil plasticity and their capacity to actively orchestrate tissue repair and regeneration. Neutrophils modulate angiogenesis, extracellular matrix (ECM) remodeling, macrophage polarization, and resolution of inflammation. Distinct neutrophil subsets—including pro-inflammatory and pro-resolving phenotypes—have been identified, highlighting their dynamic adaptation to microenvironmental cues. Notably, neutrophils contribute to vascular network formation through the release of matrix metalloproteinases, growth factors, reactive oxygen species (ROS), neutrophil extracellular traps (NETs), and specialized pro-resolving mediators (SPMs). In the context of biomaterial implantation, neutrophils are the first immune cells to interact with implanted devices, critically shaping the foreign body response (FBR) and influencing downstream regenerative outcomes. While early tissue engineering strategies aimed to suppress neutrophil recruitment, recent advances in immunomodulatory biomaterials seek instead to harness their plasticity. Approaches include promoting timely apoptosis and efferocytosis, regulating reactive oxygen species and NET formation, and directing neutrophil polarization toward pro-regenerative phenotypes. This review discusses current insights into neutrophil heterogeneity, their role in vascularization and tissue regeneration, and emerging biomaterial-based strategies designed to modulate neutrophil responses. Understanding and strategically directing neutrophil behavior may represent a pivotal step toward the development of next-generation regenerative therapies.

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### 1. Introduction

Neutrophils have long been considered short-lived, terminally differentiated immune cells, primarily responsible for pathogen clearance through mechanisms such as phagocytosis, degranulation, and production of reactive oxygen species (ROS).<sup>1,2</sup> Nonetheless, recent advances in transcriptomics and single-cell analysis have revised this perspective. Current evidence demonstrates that neutrophils possess significant phenotypic and functional versatility, with context-specific states influenced by local cytokines, metabolic factors, and hypoxic conditions.<sup>3,4</sup> Multiple distinct subsets—including pro-inflammatory, pro-resolving, immunosuppressive, and pro-angiogenic neutrophils—have now been observed in various settings such as infection, cancer, ischemia, and sterile injury models.

Beyond their antimicrobial activity, neutrophils actively participate in tissue repair. Tissue regeneration requires the coordinated interplay between immune responses, angiogenesis, extracellular matrix (ECM) remodeling, and stem/progenitor cell recruitment.<sup>1,2</sup> During the healing process, neutrophils help rebuild the ECM, remove dead cells and debris, and shape macrophage behavior through their own cell death and by being cleared away.<sup>2</sup> Crucially, neutrophils are key players in forming new blood vessels, an essential step in proper tissue healing. By releasing substances like vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), special mediators that promote healing, and neutrophil extracellular traps (NETs), neutrophils affect how endothelial cells move, how new vessels grow, and how blood vessels mature.<sup>5,6</sup>

Regenerative medicine has traditionally prioritized stem cells, growth factors, and scaffold design, often neglecting the immune system's critical role in tissue repair. Recent studies show that inflammation is a controlled and essential part of regeneration, prompting immunomodulatory strategies to shift early inflammatory responses toward healing.<sup>1,2</sup> Rapid advances in tissue engineering have improved targeted repair methods, with biomaterials playing a key role by providing

<sup>a</sup>i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal. E-mail: judite@ineb.up.pt

<sup>b</sup>INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal

<sup>c</sup>ICBAS – Instituto Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal



structural support and mimicking natural tissue environments.<sup>7,8</sup>

Biomaterial-based tissue engineering has evolved from the development of bioinert scaffolds toward the design of bioactive and immunomodulatory platforms. An implanted biomaterial or medical device is the earliest cellular mediator. Their recruitment, activation state, and persistence at the material interface critically influence subsequent macrophage polarization, fibrotic encapsulation, and vascular integration.<sup>9,10</sup> The type and profile of immune cells present at the injured site will contribute to the outcome and quality of the repair process, since these cells can create a microenvironment favorable to regeneration by secreting molecules, such as growth factors and cytokines.<sup>11–14</sup> Another fundamental factor for successful tissue regeneration is the establishment of efficient vascularization, which ensures nutrient and oxygen supply, thereby guaranteeing the long-term survival and function of implanted biomaterials and medical devices.<sup>15</sup> Therefore, the immunomodulatory, especially through the resolution of inflammation, and the pro-vascularization effects of biomaterials, are key factors in regenerative medicine.<sup>16</sup>

Current strategies sought to suppress neutrophil infiltration, but now it is increasingly recognized that precise modulation—rather than inhibition—of neutrophil activity may optimize regenerative outcomes. Recent studies demonstrate that biomaterial physicochemical properties—including stiffness, surface chemistry, topography, and degradation kinetics—directly shape neutrophil activation, NET formation, apoptosis, and polarization.<sup>17,18</sup> Furthermore, engineered systems capable of temporally controlling neutrophil recruitment and resolution have shown promising results in promoting angiogenesis, stem cell recruitment, and functional tissue repair. These findings position neutrophils as critical mediators linking early inflammation to constructive remodeling.

In this review, we discuss emerging evidence redefining neutrophils as central regulators of biomaterial-guided regeneration. We examine their plasticity, role in vascularization, and contribution to organ-specific repair, and highlight biomaterial-based strategies to modulate neutrophil apoptosis, ROS production, and polarization. By integrating advances in neutrophil biology with immunoengineering approaches, we propose that harnessing neutrophil plasticity represents a key step toward the rational design of next-generation regenerative biomaterials.

While previous reviews have highlighted the roles of neutrophils in tissue repair and regeneration,<sup>19,20</sup> our review integrates these insights with emerging evidence on neutrophil plasticity and their contributions to organ-specific repair, emphasizing implications for biomaterial-based regenerative medicine applications.

## 2. The neutrophil

Neutrophils are the most abundant cell type of the innate immune system constituting about 60% to 70% of all leuko-

cytes in human blood. Every day,  $10^{11}$  cells are produced and mature in the bone marrow. From there, neutrophils enter the bloodstream and circulate until they leave into tissues. When their lifespan ends within tissues, apoptotic neutrophils are phagocytosed mainly by macrophages.<sup>2,4,7,21–23</sup>

Neutrophils are produced from hematopoietic cells, following a differentiation process that includes five stages: promyelocyte, myelocyte, metamyelocyte, band cell, and the mature neutrophil. Immature neutrophils, characterized by banded or ring-shaped nuclei, are released from the bone marrow during stress or inflammation and exhibit enhanced inflammatory and oxidative burst capacities. Terminally differentiated neutrophils are known as polymorphonuclear (PMN) cells and their nucleus has a segmented shape, and the cellular cytoplasm contains granules and secretory vesicles that stores specific proteins relevant to their functions.<sup>3,21</sup>

### 2.1. Neutrophil dynamics and recruitment

Under homeostatic conditions, these cells can be found in the bone marrow, spleen, liver, and lungs, among other organs, and only 1–2% are in circulation. There is still scarce knowledge on how neutrophils are directed to these organs and on the functions they perform. It is believed that they may serve as reservoirs for mature neutrophils that can quickly respond to inflammation or infection, or the marginated granulocytes may be continuously monitoring these organs for tissue damage or microbial invasion.<sup>1,2,4,7,21–23</sup>

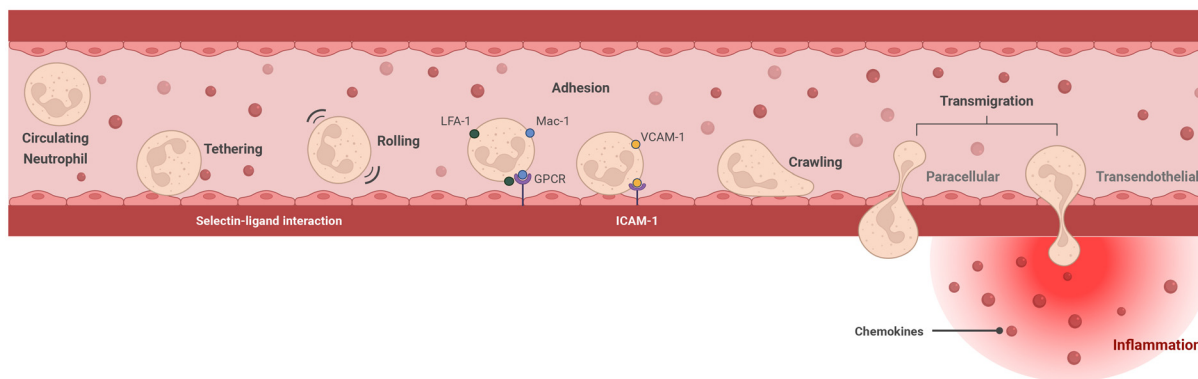
Under inflammatory conditions, neutrophils from the blood migrate to the injury site as early as 20 minutes after injury. The injured cells release danger signals, including damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs). DAMPs consist of host-derived molecules like adenosine triphosphate (ATP) and high mobility group protein B1 (HMGB1), or modified proteins such as fragmented hyaluronan or collagen. PAMPs cover bacterial and viral products such as lipopolysaccharides (LPS) and unmethylated cytosine phosphate guanosine motifs. Both initiating signals can activate the same pattern recognition receptors, which initiate neutrophil recruitment.<sup>1,2,23,24</sup>

Neutrophil recruitment to infection or injured sites is a regulated, multistep process, including tethering, rolling, adhesion, crawling, and transmigration, mainly occurring in post-capillary venules (Fig. 1). It involves an initial phase triggered by short-term signals and a subsequent amplification phase with increased infiltration driven by long-lasting signals.<sup>2,23,24</sup>

Inflammatory stimuli induce endothelial cells to express selectins, adhesion molecules, and chemokines that initiate neutrophil recruitment and rolling along the vascular wall. Rolling is mainly mediated by selectin–ligand interactions, which slow neutrophils in a shear flow and allow them to sense activating signals. Chemokines presented on the endothelial surface then trigger rapid inside-out signaling through G-protein-coupled receptors (GPCRs), leading to conformational changes in neutrophil integrins such as lymphocyte function-associated antigen 1 and macrophage antigen 1 (Mac-1).



## Neutrophil Recruitment Processes



**Fig. 1** Neutrophil recruitment. Neutrophil recruitment to sites of infection or tissue injury is a tightly coordinated process regulated by sequential adhesive and signalling interactions between circulating neutrophils and the vascular endothelium. The process begins with endothelial activation induced by inflammatory mediators, leading to the expression of selectins and adhesion molecules that mediate neutrophil tethering and rolling along the vessel wall. Chemokines presented on the endothelial surface activate neutrophil G protein-coupled receptors (GPCRs), promoting conformational activation of  $\beta 2$  integrins. This results in firm adhesion through integrin binding to endothelial ligands, followed by intraluminal crawling as neutrophils migrate along the endothelium. After transmigration, neutrophils migrate toward the sites of inflammation. Created with BioRender.com (LFA-1: lymphocyte function-associated antigen 1; Mac-1: macrophage antigen 1; GPCR: G-protein-coupled receptor; VCAM-1: vascular cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1).

This activation increases integrin affinity and avidity, resulting in firm adhesion (arrest) of neutrophils to endothelial ligands including intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).<sup>1,6,24</sup>

Following firm adhesion, neutrophils undergo adhesion strengthening and intraluminal crawling to locate permissive sites for transmigration. Transendothelial migration can occur *via* paracellular routes, through endothelial junctions, or transcellular routes, directly through endothelial cells, and is mediated by coordinated interactions between neutrophil integrins and endothelial junctional molecules such as platelet/endothelial cell adhesion molecule 1, junctional adhesion molecules, cluster of differentiation (CD) 99, and endothelial cell-selective adhesion molecule. After crossing the endothelium, neutrophils must also penetrate the basement membrane and pericyte layer, often at regions of reduced ECM density. These later stages involve additional integrins, proteolytic enzymes, and cytoskeletal rearrangements that facilitate efficient neutrophil entry into infected or damaged tissues while preserving vascular integrity.<sup>1,6,24</sup>

## 2.2. Phagocytosis and bactericidal activity

The initial role of neutrophils is the elimination of pathogens through phagocytosis, release of ROS and granule proteins, the formation of NETs and secretion of inflammatory cytokines.<sup>3,7,23,25</sup>

After the recognition of a pathogen by neutrophils, phagocytosis occurs in the phagolysosome, involving microbial engulfment. The process starts when opsonic receptors activate kinases to reorganize the cytoskeleton for internalization.<sup>26</sup> Following phagocytosis, neutrophils eliminate internalized pathogens through two interrelated antimicrobial mechanisms: oxygen-dependent and oxygen-independent pathways. The

oxygen-dependent response is initiated by the rapid activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, which assembles on the phagosomal membrane upon neutrophil activation by phagocytosis or extracellular stimuli. Assembly of the active oxidase requires phosphorylation and translocation of cytosolic subunits, including p47phox and p67phox, processes regulated by protein kinase C and mitogen-activated protein kinase signaling.<sup>26</sup>

The activated NADPH oxidase transfers electrons from cytosolic NADPH to molecular oxygen, producing superoxide anions within the phagosome. This primary ROS is subsequently converted into highly microbicidal products, including hydrogen peroxide, hydroxyl radicals, and hypochlorous acid, which collectively mediate effective pathogen killing. Although this oxidative burst is classically associated with microbial clearance, neutrophils can also generate elevated levels of ROS in sterile inflammatory conditions, such as trauma or acute liver failure, highlighting the broader role of oxidative mechanisms in inflammatory responses.<sup>3,27</sup>

Neutrophils contain specialized intracellular granules that store a wide array of bactericidal molecules and play a central role in non-oxidative antimicrobial defense. Upon pathogen encounter, these granules either fuse with phagosomes to mediate intracellular killing or are released by exocytosis to act extracellularly. Granule-derived antimicrobial agents include myeloperoxidase, lysozyme, lactoferrin, matrix metalloproteases, and several proteases, notably neutrophil elastase and cathepsin G. Among these, azurophilic granules serve as a major reservoir of serine proteases such as neutrophil elastase, cathepsin G, proteinase 3, and azurocidin, which are critical for the degradation and killing of engulfed microbes.<sup>3,27</sup>

Following phagocytosis, granule-phagosome fusion delivers these enzymes into the developing phagolysosome, where



dynamic changes in intraphagosomal pH are essential for optimal protease activity. An initial alkalinization of the phagosomal lumen, driven by superoxide anion dismutation, creates conditions that favor early protease activation and efficient microbial killing. As ROS production declines, the phagosome gradually acidifies in parallel with continued granule fusion.<sup>6,27</sup>

### 2.3. Neutrophil extracellular traps (nets): mechanisms and functional implications

In addition to conventional intracellular killing and degradation of individual bacteria, the concept of extracellular killing of bacteria by neutrophils *via* NETs has received much attention over the past decade. NETs represent an important antimicrobial mechanism that complements conventional intracellular killing by neutrophils. NETs are composed of decondensed chromatin decorated with granular and cytosolic proteins that are actively expelled into the extracellular space in response to pro-inflammatory stimuli such as interleukin (IL)-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and LPS. This process, termed NETosis, was initially described as a form of cell death occurring hours after stimulation *in vitro*. However, *in vivo* observations using intravital microscopy have demonstrated that neutrophils can remain viable after NET release, generating anuclear cells that retain functional capacities such as phagocytosis and phagosome maturation, indicating that NET formation does not inevitably result in neutrophil death.<sup>3,6,7,23,27</sup>

The molecular pathways underlying NET formation are complex and context dependent. In response to stimuli such as phorbol esters or calcium ionophores, protein kinase C activation and calcium influx promote the translocation of neutrophil elastase and myeloperoxidase from granules to the nucleus, where they drive chromatin decondensation. This process requires Rac2 activity and ROS generated by the NADPH oxidase, which activate protein arginine deiminase 4, leading to histone citrullination and chromatin unfolding. In contrast, NETosis induced by bacteria or immune complexes proceeds through a distinct signaling cascade involving integrin engagement and activation of SFKs and spleen tyrosine kinase. While NETs contribute to microbial trapping and killing, their content of nuclear and granular components may also expose autoantigens and release DAMPs, potentially amplifying inflammation and promoting tissue injury.<sup>3,6,27</sup>

Activated neutrophils can also produce immunomodulatory chemokines which coordinate further immune cell recruitment. Oxidative stress produced by neutrophils opens interendothelial junctions, thereby promoting the migration of inflammatory cells across the endothelial barrier. Once the injury site is disinfected, neutrophils secrete cytokines and growth factors to promote proliferation of nearby fibroblasts, keratinocytes, and endothelial cells.<sup>4,7,23</sup>

Negative regulatory mechanisms maintain the intricate balance between effective antimicrobial responses and excessive inflammation. Uncontrolled activation of neutrophils in an inflammatory microenvironment can lead to tissue damage

by excessive extracellular degranulation and the release of neutrophil proteases.<sup>1,3,27</sup>

A significant advance in neutrophil biology was the recognition that peripheral (not present in the bone marrow or other niches of generation) neutrophils are a heterogeneous population of cells, where immature and PMN neutrophil cells are simultaneously present in the blood.<sup>28</sup>

In recent years, the previously simplistic perspective on neutrophils has been significantly reassessed, leading to the development of several new paradigms. The application of advanced methods, including intravital microscopy, genetic fate mapping, and single-cell sequencing, has substantially advanced research in this area and facilitated deeper investigations into the complexities of neutrophil biology.<sup>2</sup>

## 3. The plasticity of neutrophils

Neutrophils have traditionally been regarded as short-lived cells, with a limited scope of action within the immune system, with a half-life of about 1.5 hours in mice and 8 hours in humans. Nevertheless, a recent study proposed that, under basal conditions, the average circulatory lifespan of neutrophils can be up to 12.5 hours in mouse cells and an unprecedented 5.4 days in human cells, challenging the concept that neutrophils are a homogeneous population of terminally differentiated cells.<sup>1,4,25,29</sup>

Recent developments in neutrophil biology have revealed the existence of distinct neutrophil subsets that comprise a spectrum of phenotypic states that have broader mechanisms of action in infection, inflammation and cancer immunology.<sup>1,4,30</sup> Furthermore, neutrophils can dynamically adjust their transcriptional and functional profiles shaped by microenvironmental cues, metabolic adaptations and epigenetic modifications.<sup>3</sup>

Neutrophil heterogeneity was first described in 1986, when low-density neutrophils (LDNs) and high-density neutrophils (HDNs) were separated using a gradient separation procedure. These two populations were easily distinguishable in the blood of patients with acute or chronic inflammatory diseases.<sup>31</sup> Different functionally neutrophil subsets were observed in cancer, where tumor-associated neutrophils (TANs) were described to exhibit polarized states of N1 (antitumor) or N2 (protumor) phenotypes, influenced by the environmental context and specific tumor-derived cytokines, similar to tumor-associated macrophages.<sup>32,33</sup> A recent study demonstrated temporal neutrophil polarization following myocardial infarction, and also suggested that, similar to macrophages, *in vitro*, peripheral blood neutrophils can be polarized to pro-inflammatory or anti-inflammatory phenotypes.<sup>33</sup> Tsuda, Y. *et al.*<sup>34</sup> observed that neutrophils that were associated with resistance to methicillin-resistant *Staphylococcus aureus* (MRSA) had a multilobular nucleus (indicative of mature cells), and the nucleus of the neutrophils associated with susceptibility to MRSA was ring-shaped. Grieshaber-Bouyer *et al.*<sup>35</sup> employed single-cell ribonucleic acid (RNA) sequencing to characterize



the neutrophil compartment across various tissues in mice. Their findings revealed a conserved neutrophil differentiation trajectory across tissues, with distinct subsets corresponding to distinct maturation stages. Additionally, they observed tissue-specific adaptations in neutrophil gene expression profiles, suggesting that the local microenvironment can shape neutrophil functional specialization.

Neutrophils display considerable heterogeneity across multiple dimensions, including: (i) nuclear morphology; (ii) polarization; and (iii) density (Fig. 2). This plasticity encompasses a continuum of differentiation stages, ranging from immature progenitors to aged or senescent neutrophils.

### 3.1. Neutrophil heterogeneity based on the nuclear morphology

Immature neutrophils—often identified by a banded nuclear morphology or low-density properties—are mobilized from the bone marrow during emergency granulopoiesis and are associated with heightened inflammatory activity and enhanced oxidative burst capacity. In contrast, aged neutrophils, characterized by increased CXC chemokine receptor type 4 (CXCR4) and reduced cluster of CD62L expression, exhibit diminished antimicrobial functions but an increased tendency toward NET formation and immunomodulatory roles.<sup>3,33</sup>

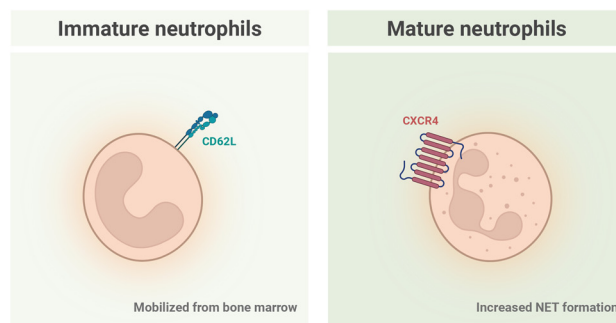
Functionally distinct subsets of neutrophils have been described to appear in specific types of tissue or injury. Under specific conditions, some mature neutrophils can undergo proliferation outside the bone marrow, and this can further enhance their persistence within tissues. Moreover, injury-mediated danger signals not only activate and recruit mature neutrophils, but also extend the lifespan of specific subsets of neutrophils.<sup>1,36</sup> Proinflammatory mediators, such as granulocyte-macrophage colony-stimulating factor, interleukin-1 beta (IL-1 $\beta$ ), and LPS, also extend their lifespan by delaying neutrophil apoptosis through the activation of the phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase pathways.<sup>3</sup> An extended neutrophil lifespan enables complex tissue roles, including inflammation resolution and immune response influence.<sup>1</sup> Nevertheless, it is important to note that upon recruitment to inflamed tissues, neutrophils can exhibit extended survival, yet their population turnover remains rapid. For example, in a muscle injury model, neutrophil influx peaked at 24 hours, followed by disappearance and emergence of reparative macrophages.<sup>37</sup> These dynamics suggest that the *in vivo* durability of neutrophil reprogramming may be limited by rapid cell turnover, and this aspect should be further explored.

### 3.2. Functional polarization of neutrophils in inflammation

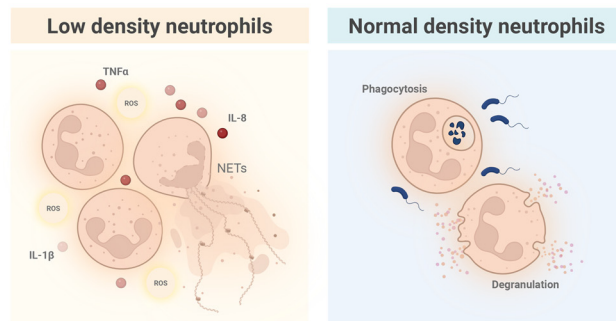
During the resolution phase of inflammation, neutrophils can undergo functional reprogramming from a pro-inflammatory to a pro-resolving phenotype. This transition is driven by local mediators such as annexin A1 and specialized pro-resolving lipid mediators (SPMs), including resolvin E1, which activate specific receptors and downstream signaling pathways that reshape neutrophil gene expression and effector functions.

## Neutrophil Heterogeneity

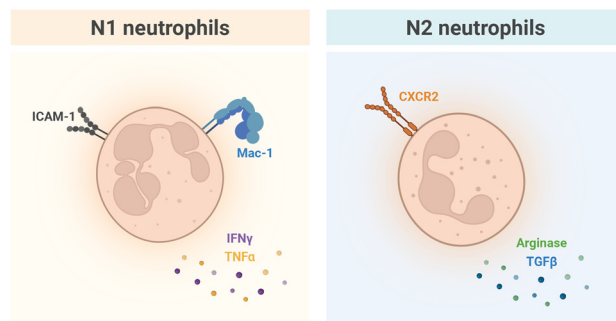
### Nuclear Morphology



### Density



### Polarization



**Fig. 2** Neutrophil heterogeneity and plasticity. Neutrophils can be characterized across multiple dimensions, including morphology, density, and polarization state. Morphological diversity encompasses variations associated with maturation, activation, and aging that influence function. Density-based subsets, identified as normal-density and low-density neutrophils, arise during inflammation and disease and display distinct immunoregulatory properties. Additionally, neutrophils shift between pro-inflammatory and pro-resolving states, contributing to both inflammation and tissue repair. These phenotypic transitions are regulated by environmental cues, cytokine signalling, and chemokine receptor engagement. Created with BioRender.com. (CD62L: Cluster of differentiation 62L; CXCR: CXC chemokine receptor; NETs: Neutrophil extracellular traps; ROS: Reactive oxygen species; IL-1 $\beta$ : interleukin-1 $\beta$ ; Mac-1: macrophage antigen 1; TNF- $\alpha$ : tumor necrosis factor  $\alpha$ ; IFN- $\gamma$ : interferon- $\gamma$ ; TGF- $\beta$ : transforming growth factor- $\beta$ ).



Hypoxic microenvironments in inflamed or neoplastic tissues stabilize hypoxia-inducible factor-1 alpha, driving a metabolic shift toward glycolysis and also promoting a pro-inflammatory phenotype. Pro-resolving neutrophils are characterized by enhanced apoptosis, increased production of anti-inflammatory cytokines such as interleukin-10 (IL-10), and the release of growth factors and proangiogenic mediators that promote tissue repair and restoration of homeostasis.<sup>3</sup>

Drawing a parallel with the classical M1–M2 polarization paradigm described for macrophages, neutrophils have also been proposed to adopt functionally distinct N1 and N2 phenotypes. Although this binary classification likely oversimplifies the complexity of neutrophil plasticity, inflammatory mediators such as cytokines, chemokines, PAMPs, and DAMPs are recognized as key drivers of neutrophil polarization and functional reprogramming.<sup>3,4,29,30</sup> Proinflammatory stimuli, including LPS, TNF- $\alpha$ , and interferon- $\gamma$  (IFN- $\gamma$ ), promote the acquisition of an N1 phenotype, whereas anti-inflammatory signals such as transforming growth factor- $\beta$  (TGF- $\beta$ ), interleukin-4 (IL-4), and glucocorticoids favor N2 differentiation.<sup>3,29</sup>

Phenotypically, N1 neutrophils exhibit elevated expression of activation markers such as CD11b, CD66b, CD64, and the chemokine receptor type 2 (CXCR2), facilitating their recruitment to sites of inflammation. In contrast, N2 neutrophils express higher levels of CD16, CD163, CD206, and CXCR4, consistent with immunoregulatory functions, homing to the bone marrow or lymphoid organs, and roles in wound healing and tissue remodeling.<sup>3,4</sup> Beyond their direct effector functions, N1 and N2 neutrophils may also influence immune responses indirectly by modulating macrophage polarization toward M1 or M2 phenotypes, respectively.<sup>1</sup>

### 3.3. Density-based neutrophil subsets and functional diversity

Neutrophil heterogeneity further encompasses the differences in buoyancy, referring to the separation of cells based on their density during gradient centrifugation. Accordingly, LDNs co-isolate with peripheral blood mononuclear cells, whereas normal-density neutrophils (NDNs) are recovered in the granulocyte fraction, with both populations exhibiting distinct phenotypic and functional characteristics.<sup>3</sup> LDNs include granulocytic myeloid derived suppressor cells (G-MDSC) and neutrophils with immunosuppressive properties.<sup>21</sup> LDNs and G-MDSC cells share multiple morphological and phenotypic features with mature neutrophils, including segmented nuclei and overlapping cell-surface marker expression. These neutrophil-like populations have been implicated—albeit controversially—in cancer and other inflammatory conditions. Although it has been proposed that TAN1 and TAN2 phenotypes may preferentially derive from NDNs and LDNs, respectively, this relationship remains speculative.<sup>38</sup>

G-MDSC cells represent one of the best-characterized subsets and are consistently associated with immune suppression across disease contexts.<sup>4</sup> These cells inhibit T and natural killer cell proliferation and can be isolated together with monocytes by density gradient centrifugation. In cancer

patients, G-MDSCs are characterized by the expression of CD11b, CD66b, and high levels of CD15, while lacking CD14, and are enriched within the LDN fraction of peripheral blood mononuclear cells. Notably, a population of CD62L-expressing neutrophils within the MDSC compartment has been shown to suppress T-cell responses through ROS release, expanding significantly in tumor-bearing hosts compared with healthy individuals.<sup>25</sup>

Functionally, LDNs are enriched under inflammatory and pathological conditions and display an activated yet immunosuppressive phenotype, characterized by elevated ROS production, proinflammatory cytokine secretion, and NET formation, alongside increased expression of adhesion molecules and chemokine receptors. Despite this activated profile, LDNs exhibit impaired phagocytic capacity compared with NDNs. In contrast, NDNs predominate in healthy individuals and display robust antimicrobial functions, including efficient phagocytosis and degranulation.<sup>3</sup>

We observed a lack of coherence in neutrophil nomenclature throughout the available literature, namely in what regards HDN and NDN. Recent studies have suggested that the term “high-density neutrophils” may be misleading and that these cells should instead be referred to as “normal-density neutrophils”, reflecting their physiological prevalence and functional competence.<sup>25</sup> There is a clear need to standardize the nomenclature used.

Despite the increasing efforts to delineate neutrophil subsets, the developmental relationship between LDNs and NDNs remains unresolved. Some studies suggest that LDNs represent a distinct lineage, whereas others propose that they arise from the activation, priming, or aging of NDNs.<sup>3</sup> More broadly, it remains unclear whether neutrophil heterogeneity reflects the existence of discrete lineages or instead originates from a single, highly adaptable precursor capable of responding dynamically to local environmental cues.<sup>1,2</sup>

Given the substantial overlap in the morphology, phenotype, and immunomodulatory capacity among LDNs, MDSCs, TANs, and mature neutrophils, it is plausible that these populations represent a continuum of functional states rather than distinct cell types. According to this view, neutrophil heterogeneity arises from remarkable cellular plasticity, allowing rapid adaptation to microenvironmental stimuli and cell–cell interactions. Differential mobilization of cytoplasmic granules and stimulus-dependent exposure of granule-associated membrane proteins may further alter the neutrophil surface marker composition, potentially leading to the erroneous identification of novel subsets.<sup>38</sup>

Despite the growing evidence supporting the existence of neutrophil subsets in diverse experimental and disease settings, their functional relevance remains incompletely defined. The observed heterogeneity does not consistently translate into clearly delineated functional programs and the molecular mechanisms governing subset generation, stability, and regulation remain largely unexplored.<sup>3,4,33</sup> Moreover, although phenotypic plasticity and subset interconversion are well documented in monocytes and macrophages, the short



lifespan of neutrophils raises important questions regarding the durability of their reprogramming. Nonetheless, emerging evidence suggests that neutrophil functional states can be therapeutically manipulated; for example, activation of peroxisome proliferator-activated receptor gamma has been shown to skew neutrophils towards an N2-like phenotype, leading to improved outcomes in experimental models of stroke.<sup>6</sup>

## 4. Neutrophils and vascularization

The processes of vascularization and inflammation are mutually dependent; there is an actual interplay between these cell types. In fact, the extravasation of immune cells from blood vessels to tissues is mediated by endothelial cells that express several leukocyte adhesion molecules, whereas immune cells secrete soluble factors that will have an effect on endothelial cell proliferation, migration and activation and are able to create a microenvironment that will lead to increased tissue vascularization.<sup>39</sup>

Vascularization and angiogenesis are an essential support for healing because the new vasculature delivers oxygen and nutrients that facilitate tissue regrowth.<sup>2</sup> Key angiogenic cytokines include VEGF, angiopoietin, fibroblast growth factor (FGF), and TGF- $\beta$ , with VEGF playing a central role in vessel formation and directly attracting endothelial cells.<sup>6</sup> Cells of the immune system are key in the process of new vessel formation, and their ability to polarize towards different phenotypes makes them able to either induce or inhibit the process of angiogenesis through the production of several different factors.<sup>40</sup>

Mature neutrophils contain more than 700 proteins, including growth factors or pro-angiogenic factors, stored in the nucleus and granules. These proteins can be quickly released upon activation and directly contribute to the reconstruction of the vascular network.<sup>5,6</sup> Several studies highlight the important role of neutrophils in vascularization. In a rodent wound healing model using knockout animals with defective neutrophil recruitment, a decrease in neovascularization was observed.<sup>41</sup> Also in a rodent wound healing model, using CD18-deficient mice that show impaired neutrophil infiltration, vascularization was significantly affected when compared with the wild-type animals.<sup>42</sup> In another study with implants containing assembled human vascular networks, the depletion of neutrophils using lymphocyte antigen 6 complex locus G6D ( $\alpha$ -Ly6G) antibodies leads to impaired vascularization, and the subsequent transfer of neutrophils restored vascularization.<sup>30</sup> In a murine model of brain ischemia, the depletion of neutrophils inhibited VEGF-induced angiogenesis.<sup>43</sup> Using an animal model of oxygen-induced retinopathy neutrophil depletion caused a decrease in neovascularization.<sup>44</sup> In an *ex vivo* model of colitis-induced murine colorectal cancer, the vessel density was evaluated after neutrophil depletion and a decrease in vessel density was observed, confirming the importance of neutrophils in tumor vascularization.<sup>45</sup> The pro-angiogenic role of neutrophils has also been validated in advanced

co-culture systems. Herath *et al.* established a triple-cell *in vitro* model comprising human umbilical vein endothelial cells (HUVECs), human osteoblasts (HOBs), and neutrophils to investigate angiogenesis and osteogenesis over 14 days. Neutrophils significantly enhanced the formation of stable microvessel-like networks and upregulated pro-angiogenic markers as vascular endothelial growth factor A (VEGF-A), CD34, epidermal growth factor, as well as several osteogenic markers, in a dose- and time-dependent manner, supporting their regenerative potential beyond early inflammatory defense.<sup>46</sup>

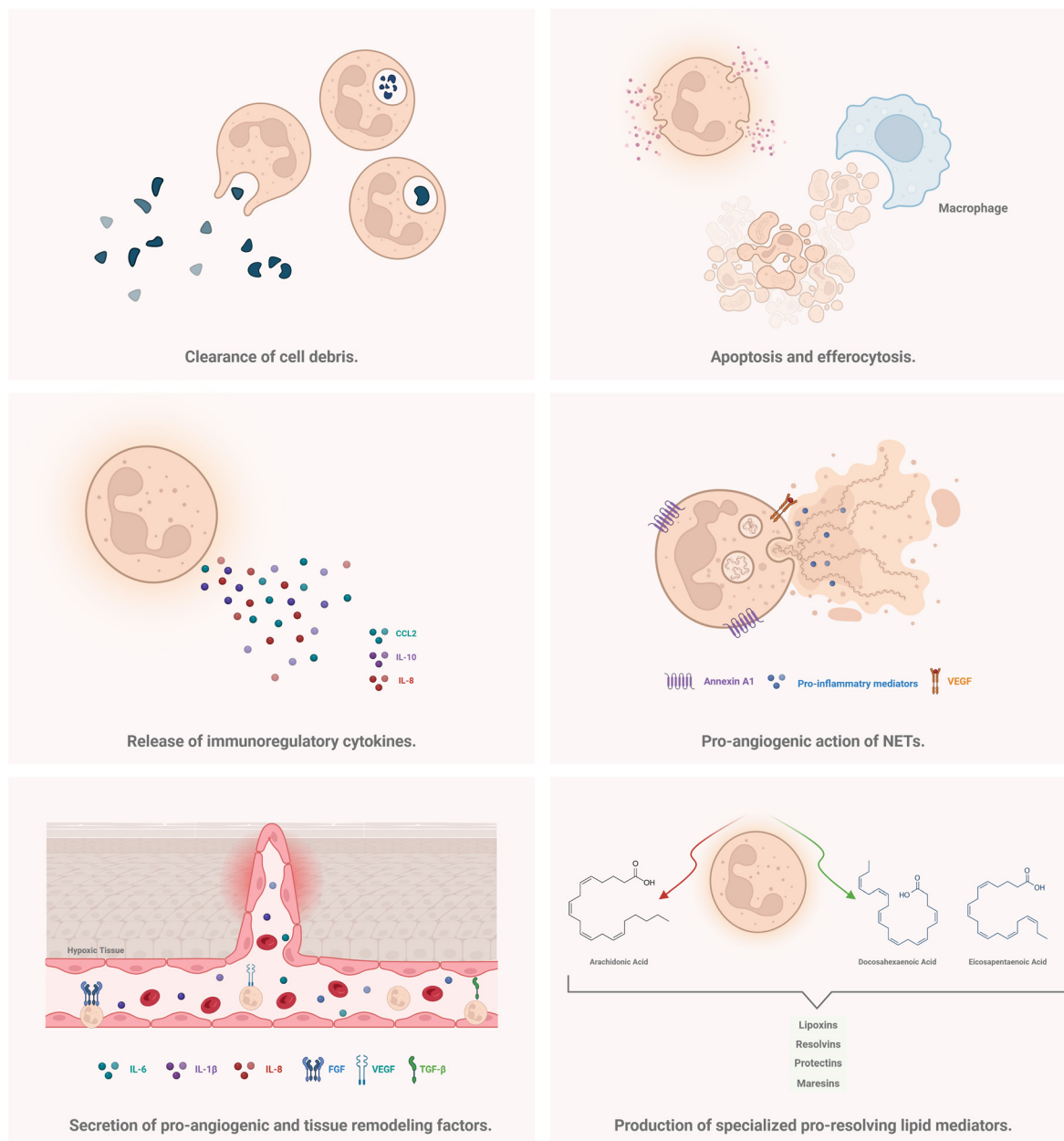
Neutrophils are one of the essential immune cells involved in promoting vascular network construction, adopting different subtypes according to the inflammatory microenvironment that they encounter.<sup>20</sup> Neutrophils mediate tissue revascularization through different mechanisms (Fig. 3): (i) breakdown of damaged cells and the ECM with lytic and proteolytic enzymes; (ii) clearance of cell debris through phagocytosis; (iii) apoptosis and subsequent efferocytosis performed by macrophages; (iv) release of immunoregulatory cytokines which govern the activities of macrophages and T helper cells; (v) secretion of pro-angiogenic and tissue remodeling factors; (vi) pro-angiogenic action of NETs and ROS; (viii) production of SPMs.

Neutrophil apoptosis, and subsequent clearance by macrophages, is the most studied mechanism of tissue regeneration.<sup>5,6</sup> This process of cell death preserved the integrity of the plasma membrane, which elicits anti-inflammatory and pro-resolution responses.<sup>47</sup> The apoptotic cells release low amounts of nucleotides, ATP and uridine triphosphate which act as 'find me' signals. These soluble mediators bind to the macrophage purinergic receptor P2Y2, while the lipid mediators lysophosphatidylcholine (LPC) and sphingosine 1-phosphate (S1P) bind to macrophage receptors G2A and S1P1-5, respectively.<sup>1,3,4,47</sup> After recognition by macrophages, apoptotic neutrophils undergo efferocytosis. The cells release 'eat me' signals, such as membrane lipid phosphatidylserine (PS) and calreticulin, on their surface that are detectable by macrophage receptors and trigger actin rearrangements and subsequent engulfment of the dead cells.<sup>1,3</sup> By promoting their own clearance, neutrophils play a pivotal role in resolving inflammation and induce a phenotypic change on macrophages to an M2 anti-inflammatory profile. M2 macrophages release anti-inflammatory cytokines and pro-resolving lipid species, which facilitate the subsequent process of revascularization and tissue regeneration.<sup>3</sup>

Pro-angiogenic neutrophils (PANs), which display anti-inflammatory and pro-resolving properties, have been identified in humans and mice, and it was observed that the inhibition of the recruitment of PANs to hypoxic tissues impairs vessel formation.<sup>48,49</sup> Circulating pro-angiogenic neutrophils migrate to hypoxic tissues that are rich in VEGF-A such as tumors, transplants and myocardial infarcts, and they are crucial for the initiation of angiogenesis because they deliver the pro-angiogenic enzyme MMP-9.<sup>50</sup> MMP-9 can degrade DAMPs such as HMGB1 and heat shock protein 90, dampening the recruitment of additional inflammatory cells, and is a



## Neutrophil Mediated Tissue Repair Mechanisms



**Fig. 3** Neutrophil-mediated tissue repair mechanisms. Neutrophils contribute to tissue repair and resolution of inflammation through various mechanisms. Upon tissue injury, neutrophils promote clearance of cellular debris and apoptotic cells, facilitating restoration of tissue homeostasis. Apoptotic neutrophils undergo efferocytosis by macrophages, which supports the transition toward a pro-resolving immune environment. Moreover, they also release immunoregulatory cytokines. The formation of neutrophil extracellular traps (NETs) can exert pro-angiogenic effects by providing structural and molecular cues that stimulate vascular growth. Additionally, neutrophils secrete pro-angiogenic factors that promote revascularization and extracellular matrix (ECM) remodelling. Finally, neutrophils contribute to the production of specialized pro-resolving lipid mediators (SPMs), which promote inflammation resolution and tissue regeneration. Created with BioRender.com (CCL: chemokine ligand; IL: interleukin; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; TGF- $\beta$ : transforming growth factor- $\beta$ ).

potent activator of VEGF activity.<sup>2</sup> In a model of avascular pancreatic islet transplantation in mice, MMP-9 was shown to be essential in the revascularization of transplanted islets.<sup>51</sup> The release of MMPs by neutrophils facilitates the degradation of

the provisional ECM, enabling endothelial cells, fibroblasts, and keratinocytes to migrate and proliferate.<sup>3</sup>

Angiogenesis is also promoted by neutrophils through the secretion of pro-angiogenic cytokines, directly stimulating



angiogenesis, fibroblast activation, and ECM deposition, such as VEGF, angiopoietin, FGF, and TGF- $\beta$ . Moreover, hepatocyte growth factor (HGF) and fibroblast growth factor 2 (FGF-2) further stimulate endothelial cell proliferation and tube formation.<sup>3,5</sup>

The proliferative phase is a dynamic and time-dependent process characterized by a biosynthetic switch from pro-inflammatory mediators to pro-resolving lipid mediators. During this transition, infiltrating neutrophils shift their lipid mediator profile toward SPM production, thereby actively contributing to the termination of the inflammatory response. SPMs are bioactive lipid mediators derived from polyunsaturated fatty acids, such as arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and n-3 docosapentaenoic acid (n-3 DPA), and include lipoxins, resolvins, protectins, and maresins.<sup>52</sup> SPMs exert potent anti-inflammatory and pro-resolving effects by inhibiting further neutrophil recruitment, reducing vascular permeability, promoting neutrophil apoptosis, and enhancing efferocytosis, ultimately facilitating tissue repair. These biological actions are mediated through specific GPCRs which have been proposed as potential therapeutic targets in inflammatory conditions.<sup>3,52</sup> In addition to their role in resolving inflammation, neutrophil-derived SPMs, such as lipoxin A4 and resolvin D1, have been shown to enhance endothelial cell proliferation, migration, and tube formation, thereby supporting the later stages of angiogenesis and tissue regeneration.<sup>3</sup>

The toxic substances produced by neutrophils, such as NETs, oxidants, and proteases, may also contribute significantly to the revascularization process.<sup>53</sup> NETs induced the increase of capillary tube length using a two-dimensional matrigel tube assay and also lead to an increase in spheroid sprouting in a three-dimensional (3D) spheroid sprouting assay model, suggesting a pro-angiogenic effect of NETs.<sup>53</sup> In a mouse model of an induced liver injury, neutrophils facilitate liver repair by promoting the conversion of macrophages to a pro-resolving phenotype through the production of ROS.<sup>54</sup> In an *in vitro* study with human PMNs it was found that PMN-derived serine proteases are involved in the proteolytic process of HGF, a potent mediator of angiogenesis.<sup>55</sup>

Furthermore, a recent study using isolated human neutrophils cocultured with macrophages *in vitro* revealed that TNF- $\alpha$  stimulation generated microvesicles from neutrophils, exposing phosphatidylserine and annexin A1, which in turn induced macrophage release of TGF- $\beta$ .<sup>56</sup> Microvesicles are extracellular membrane vesicles initially identified as a mechanism of cell-cell communication, but the recent data also expose their involvement in the pro-resolving role of neutrophils. Release of microvesicles exposes phosphatidylserine on the cell surface, which promotes anti-inflammatory cytokine release by macrophages and dendritic cells. Annexin A1 is a pro-resolving protein stored in large amounts in neutrophil granules, and its exposition on the cell surface leads to neutrophil apoptosis, which increases the capacity of macrophage efferocytosis and the subsequent revascularization cascade.<sup>2</sup>

## 5. The potential of neutrophils in regenerative processes

Neutrophils play key roles in maintaining the balance within many organ tissues and, similar to macrophages, adopt features tailored to the needs of those tissues. They work with both innate and adaptive immune cells to guide immune responses, are involved in chronic inflammatory conditions, and exhibit regenerative characteristics, contributing to tissue repair in different organs.<sup>2,49,57–59</sup>

Neutrophils contribute to tissue repair *via* multiple mechanisms. Some reparative functions, such as MMP delivery, appear to be nonspecific and occur in all organs, while in certain situations, including healing of the optic nerve, or regrowth of lung epithelial cells, there are clear specific effector functions.<sup>2</sup> Altogether, these findings challenge the traditional view of neutrophils as purely pro-inflammatory cells and highlight their emerging role as critical regulators of tissue regeneration. The following sections describe in detail the mechanisms by which neutrophils contribute to regenerative processes in different tissues.

### 5.1. Liver

Constantly exposed to gut-derived components such as food antigens and microbial products *via* the portal vein, the liver maintains a state of tolerance under physiological conditions. However, excessive stimulation can trigger inflammation, contributing to the progression of chronic liver diseases.<sup>60</sup> Neutrophils have traditionally been associated with tissue damage during inflammation, yet recent evidence highlights their reparative roles in both acute and chronic liver injury. Intravital imaging studies demonstrated that neutrophils not only clear cellular debris, but also promote vascular regrowth by forming structural sleeves for new blood vessels.<sup>2</sup>

Neutrophils contribute to liver regeneration through multiple interconnected mechanisms. One of their primary roles is the degradation of fibrotic components *via* metalloproteinases (matrix metalloproteinase-8 (MMP-8) and MMP-9), which reduce collagen accumulation and promote fibrolysis. Experimental models of chronic liver injury have shown that increased neutrophil counts correlate with reduced fibrosis, while neutrophil depletion exacerbates fibrotic progression, highlighting the anti-fibrotic potential of neutrophils in chronic liver disease.<sup>61</sup>

Beyond fibrolysis, neutrophils orchestrate macrophage phenotype switching during liver repair. In acetaminophen-induced liver injury, neutrophil-derived ROS, primarily generated through NADPH oxidase 2 (Nox2), activate the Ca<sup>2+</sup>-CaMKK $\beta$ -AMPK pathway in macrophages. This signaling drives their conversion from a pro-inflammatory to a reparative phenotype, accelerating inflammation resolution and hepatocyte regeneration. Conversely, neutrophil depletion or Nox2 deficiency delays recovery, whereas adoptive transfer of wild-type neutrophils restores repair processes.<sup>54</sup> Additional molecular mediators reinforce these reparative functions.



MicroRNA-223, abundant in neutrophils, regulates oxidative stress and inflammatory signaling by suppressing inducible nitric oxide synthase and Nod-like receptor protein 3 expression in macrophages, promoting an M2 reparative phenotype. SPMs further limit neutrophil chemotaxis, inhibit cytokine production, and enhance phagocytosis, preventing hepatocyte apoptosis under stress conditions.<sup>60</sup>

Recent evidence also reveals a bidirectional hepatocyte–neutrophil communication network mediated by the leukemia inhibitory factor receptor (LIFR). Upon liver injury, LIFR activates signal transducer and activator of transcription 3 (STAT3)-dependent secretion of CXC chemokine ligand 1 (CXCL1) and cholesterol, recruiting neutrophils that subsequently release HGF to stimulate tissue regeneration. This interaction occurs through the LIFR–STAT3–CXCL1–CXCR2 axis and the LIFR–STAT3–cholesterol–estrogen-related receptor alpha (ERR $\alpha$ )–HGF axis.<sup>20</sup>

Finally, in focal necrotic injury models, neutrophils rapidly infiltrate damaged areas, dismantle necrotic sinusoids, and facilitate angiogenesis. ROS and hydrogen peroxide produced by neutrophils polarize macrophages toward a reparative phenotype. Interestingly, platelet C-type lectin-like receptor 2 expression modulates neutrophil recruitment, with its ablation enhancing debris clearance and overall healing.<sup>49</sup>

Collectively, these findings demonstrate that neutrophils play a dual role in liver injury, acting as both inflammatory mediators and key drivers of tissue repair. Through mechanisms involving ROS signaling, metalloproteinases, microRNAs, and intercellular crosstalk, neutrophils contribute to fibrosis resolution and regeneration. Understanding these processes may open new therapeutic avenues for the treatment of chronic liver diseases.

## 5.2. Cardiovascular system

Acute myocardial infarction (AMI) remains a leading cause of morbidity and mortality in Europe and is characterized by ischemia-induced deprivation of oxygen and nutrients, resulting in extensive cardiomyocyte death and the activation of a robust inflammatory response that initiates cardiac repair mechanisms.<sup>62</sup>

Neutrophils are among the earliest immune cells recruited to the heart following myocardial infarction, and growing evidence indicates that beyond their role in inflammation resolution, neutrophils exhibit pro-reparative and pro-angiogenic functions that are beneficial for post-infarction cardiac remodeling.<sup>49,59</sup> After MI, disruption of CXC chemokine ligand 12 (CXCL12)–CXCR4 signaling promotes neutrophil mobilization from the bone marrow to the peripheral blood and subsequent infiltration into the ischemic myocardium. Within the infarcted tissue, neutrophils participate in the clearance of necrotic cardiomyocytes and cellular debris, thereby facilitating wound healing. The phagocytosis of apoptotic neutrophils by macrophages induces the production of anti-inflammatory and pro-resolving mediators, including TGF- $\beta$ 1, IL-10, VEGF, and SPMs, which collectively promote inflammation resolution and cardiac repair. Furthermore,

dying neutrophils release antimicrobial  $\alpha$ -defensins that enhance the macrophage phagocytic capacity while attenuating their pro-inflammatory cytokine production.<sup>59</sup>

Experimental studies have demonstrated that chronic neutrophil depletion using anti-Ly6G antibodies exacerbates cardiac dysfunction, increases profibrotic cytokine expression, and augments myocardial fibrosis following AMI.<sup>63</sup> These findings highlight the importance of timely neutrophil apoptosis and efferocytosis in promoting macrophage polarization toward a reparative phenotype with an enhanced debris-clearing capacity. Moreover, a subset of recruited neutrophils undergoes phenotypic and transcriptomic reprogramming within the infarcted myocardium, giving rise to N2-like reparative neutrophils that express anti-inflammatory mediators such as arginase-1, IL-10, and TGF- $\beta$ 1. Neutrophils, in cooperation with macrophages, also release oncostatin M, which directly acts on cardiac fibroblasts and cardiomyocytes to stimulate angiogenesis and facilitate the removal of necrotic tissue.<sup>49</sup>

Angiogenesis is a critical component of effective cardiac repair after AMI and neutrophils contribute to this process as the primary source of annexin A1 in the infarcted heart, which promotes macrophage polarization toward a pro-angiogenic phenotype that supports vessel formation through VEGF-A secretion.<sup>59</sup>

ROS also play a crucial role in cardiac healing following AMI by promoting the clearance of dead cells and cellular debris. Although excessive ROS production by PMNs can be detrimental during the acute ischemic phase, accumulating evidence indicates that neutrophil-derived signals can also exert reparative effects by recruiting and activating mononuclear cells. These beneficial effects are mediated, at least in part, through macrophage polarization toward a reparative phenotype, a process involving neutrophil gelatinase-associated lipocalin. Consistent with the importance of the redox balance in post-infarction repair, antioxidant-based strategies have been explored, and coenzyme Q10 has been shown to reduce the infarct size, inflammatory burden, and oxidative stress, while improving left ventricular function following AMI.<sup>64</sup>

In line with these observations, studies using chronic myocardial infarction models induced by permanent left anterior descending coronary artery ligation have demonstrated that neutrophil depletion leads to impaired cardiac function, increased fibrosis, and elevated biomarkers associated with heart failure. These detrimental effects were associated with reduced expression of the efferocytosis receptor MerTK on cardiac macrophages, resulting in compromised clearance of apoptotic cardiomyocytes. Neutrophil-derived factors, particularly neutrophil gelatinase-associated lipocalin, were shown to induce MerTK expression and enhance the macrophage efferocytic capacity both *in vitro* and *in vivo*.<sup>62</sup>

Altogether, these findings support the concept that, beyond their classical pro-inflammatory role, neutrophils are pivotal regulators of inflammation resolution, tissue repair, and cardiac remodeling after myocardial infarction, thereby exerting a sustained impact on cardiac function.<sup>62</sup>



### 5.3. Lung

The lung is a distinctive organ with respect to neutrophil trafficking, as it harbors high numbers of neutrophils even under homeostatic conditions. Unlike other tissues, neutrophil recruitment in the lung occurs not only through high endothelial venules in a  $\beta$ 2-integrin-dependent manner, but also within the alveolar capillary bed *via* L-selectin- and  $\beta$ 2-integrin-independent pathways.<sup>65</sup>

Under pathological conditions, excessive neutrophil migration contributes to marked anatomical and functional damage, including disruption of intercellular junctions, epithelial apoptosis, denudation, and increased epithelial permeability. This injury phase is subsequently followed by a repair phase characterized by epithelial cell proliferation and re-epithelialization, which are critical determinants of recovery and survival in acute lung injury.<sup>33,66</sup> Despite their injurious potential, accumulating evidence indicates that neutrophil accumulation also contributes to lung epithelial repair and regeneration.<sup>33</sup>

Beyond their antimicrobial functions, pulmonary neutrophils exhibit features consistent with tissue repair.<sup>49</sup> During the resolution phase, neutrophils undergo apoptosis while preserving the membrane integrity to prevent the release of cytotoxic intracellular contents. Apoptotic neutrophils are subsequently cleared by alveolar macrophages, a process that induces macrophage phenotypic reprogramming toward an anti-inflammatory and pro-resolving state, characterized by reduced production of proinflammatory cytokines and increased secretion of anti-inflammatory mediators.<sup>67</sup> Transcriptomic analyses have revealed that lung neutrophils express angiogenic factors, including *Apelin* and *Vegfa*, suggesting a pro-angiogenic phenotype. Consistent with this, depletion of neutrophils in juvenile mice impaired pulmonary endothelial cell proliferation and disrupted normal lung development, highlighting a critical role for neutrophils in vascular and tissue homeostasis.<sup>49</sup> Similarly, experimental induction of neutropenia in models of acid aspiration and ventilator-induced lung injury resulted in worsened outcomes, characterized by delayed re-epithelialization and reduced compensatory epithelial cell proliferation, underscoring the importance of neutrophils in effective lung repair.<sup>67</sup>

Mechanistically, neutrophil transmigration itself can directly activate epithelial repair pathways. Studies have demonstrated that neutrophil migration across lung epithelial cells activates the Wnt/ $\beta$ -catenin signaling pathway both *in vivo* and *in vitro*, thereby stimulating epithelial regeneration.<sup>2,66</sup> A population of Wnt-responsive alveolar type II epithelial cells has been identified as a key driver of alveolar regeneration, and activation of Wnt signaling promotes the release of cysteine-rich protein 61 (Cyr61), which further supports epithelial repair. In addition, neutrophils can transfer microRNAs to epithelial cells, notably miRNA-223, which attenuates lung injury by repressing PARP-1-dependent inflammatory pathways.<sup>2,68</sup>

Neutrophil heterogeneity further contributes to their role in lung tissue repair. It was found that a subset of neutrophils

(CD11b<sup>high</sup>CD16<sup>high</sup>CD62L<sup>low</sup>) has immunoregulatory properties, suppresses T-cell activation and is considered part of the G-MDSC pool. Although virtually absent in healthy individuals, this population emerges during ALI and may function as a negative feedback mechanism to limit excessive inflammation. Notably, macrolide antibiotics such as clarithromycin have been shown to expand this subset in sepsis models, suggesting that the immunomodulatory effects of macrolides in lung injury may, in part, be mediated through G-MDSC. Neutrophils can also undergo functional polarization towards as the anti-inflammatory profile (N2) in response to extracellular cues, including angiotensin II and TGF- $\beta$ . The N2 neutrophils secrete growth factors and MMPs, such as VEGF and MMP-9, which are directly implicated in lung repair processes. MMPs cleave ECM components, modulate cell-matrix and cell-cell interactions, facilitate epithelial cell migration during re-epithelialization, and contribute to the resolution of inflammation through processing of proinflammatory mediators. Furthermore, neutrophils promote resolution through the synthesis of SPMs, including lipoxin A4, resolvins, and protectins, which inhibit late-stage neutrophil recruitment by interfering with chemotactic signaling.<sup>67</sup>

ROS generated by NADPH oxidase, classically associated with antimicrobial defense, also play a protective role in lung inflammation. NADPH oxidase-deficient mice develop exaggerated and persistent lung inflammation, increased nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) activation, and elevated proinflammatory cytokine levels following inflammatory challenges. This protective effect is mediated, at least in part, by activation of the redox-sensitive nuclear factor erythroid 2-related factor 2. Additionally, inflammatory stimuli such as intratracheal LPS induce  $\beta$ -catenin signaling in alveolar type II cells *via* elastase-mediated cleavage of E-cadherin, thereby promoting epithelial repair.<sup>2</sup>

Consistent with these findings, neutrophil elastase has emerged as a key regulator of lung epithelial repair. Elastase activity modulates critical growth factors, including TGF- $\alpha$  and TGF- $\beta$ , and promotes epithelial regeneration through E-cadherin cleavage and subsequent activation of  $\beta$ -catenin signaling. Both *in vitro* and *in vivo* studies have demonstrated that inhibition of elastase or  $\beta$ -catenin signaling impairs neutrophil-induced epithelial proliferation, whereas activation of  $\beta$ -catenin enhances alveolar epithelial repair. Subsequent work further identified Wnt-induced secreted protein and Cyr61 as essential  $\beta$ -catenin target genes required for lung epithelial regeneration following inflammation and injury. Moreover, a Wnt5a/protein kinase C signaling axis has been shown to regulate  $\beta$ -catenin co-activator interactions, promoting the differentiation of alveolar epithelial type II cells into type I-like cells, thereby contributing to restoration of the alveolar structure and function.<sup>69</sup>

### 5.4. Nervous system

Regeneration after injury to the peripheral or central nervous system (PNS or CNS) requires the immune system, and numerous studies have investigated the role of neutrophils following



injury to the brain, spinal cord or peripheral nerves.<sup>2,49</sup> Leow-Dyke *et al.*<sup>70</sup> showed that neurons could release a cluster of cytokines (CXCL1, TNF- $\alpha$ , and IL-6) to promote the infiltration of neutrophils across the endothelial monolayer. The recruitment of neutrophils to injured brain tissue allows debris clearance, protection against potential infection of the exposed parenchyma, and support for tissue regeneration.<sup>71</sup>

Following spinal cord injury (SCI), neutrophils exert protective and reparative functions by modulating the neuroinflammatory environment and promoting tissue repair. Experimental evidence indicates that neutrophils activate astrocytes, thereby contributing to blood–brain barrier reformation, preservation of locomotor function, reduction of lesion size, and protection of neuronal axons.<sup>71</sup> In this context, secretory leukocyte protease inhibitor (SLPI), produced by both neutrophils and astrocytes within the injured spinal cord, has been identified as a key mediator with beneficial effects in SCI.<sup>2</sup> Similarly, in peripheral nerve injury models, such as sciatic nerve damage, neutrophils facilitate optimal nerve repair through the timely clearance of myelin debris. In addition, specific neutrophil populations have been shown to support neuronal regeneration, as demonstrated in optic nerve injury models where neutrophil-derived oncomodulin promotes neuronal survival and axonal regrowth following inflammatory stimulation.<sup>49,71</sup>

Neutrophils also contribute to neurorepair by promoting angiogenesis and vascular remodeling at sites of neural injury. They serve as an autocrine source of vascular VEGF, which regulates neutrophil migration and directly supports angiogenesis. Newly formed blood vessels, in turn, provide essential neurogenic mediators, including brain-derived neurotrophic factor and VEGF, that support neuronal survival and regeneration. Furthermore, chemokines and proteases that drive neutrophil recruitment—such as CXCL1, CXC chemokine ligand 8 (CXCL8), granulocyte chemotactic protein-2, MMP-8 and MMP-9—are closely associated with vascular growth and remodeling, underscoring the coordinated relationship between neutrophil infiltration and angiogenesis in neural repair.<sup>71</sup>

In addition to angiogenic mediators, neutrophils secrete a range of immunomodulatory factors that directly support neuronal regeneration and survival. Upon activation, neutrophils can release transforming TGF- $\beta$ , which promotes nerve regeneration by stimulating nerve growth factor (NGF) production and by regulating astrocyte activation and scar formation following injury. Neutrophils also produce granulocyte colony-stimulating factor (G-CSF), HGF, NGF, and neurotrophin-4, all of which contribute to neuronal survival and repair. Moreover, cytokines and enzymes that modulate neutrophil function, including arginase and midkine, have been shown to enhance neurite outgrowth and prevent neuronal cell death, further highlighting the multifaceted role of neutrophils in nervous system repair.<sup>71</sup>

### 5.5. Skin

Following skin injury, neutrophil recruitment to necrotic wound sites is guided by multiple molecular cues that are

essential for efficient wound closure, as disruption of these signals delays healing. Formylated peptides originating from necrotic host cell mitochondria or translocated commensal bacteria act as early chemoattractants for neutrophils. In addition, MMP-8-mediated collagen degradation generates the tripeptide proline–glycine–proline, which promotes neutrophil migration; *in vivo* studies using MMP-8-deficient mice indicate that neutrophil recruitment to skin wounds is partially dependent on this pathway. Upon arrival at the injury site, neutrophils initiate a reparative cascade by coordinating the activity of resident and recruited immune and stromal cells. Notably, neutrophil-derived CXC chemokine ligand 10 (CXCL10) recruits plasmacytoid dendritic cells that facilitate commensal microbial clearance in open wounds, while CXCL10 complexed with bacterial deoxyribonucleic acid (DNA) activates wound-healing programs in neighboring macrophages and fibroblasts. In parallel, apoptotic neutrophils release lysophosphatidylserine, which is sensed by type 3 innate lymphoid cells *via* G-protein-coupled receptor 34, leading to innate lymphoid Cells group 3 activation and promotion of epithelial repair.<sup>49</sup>

Although neutrophils are key mediators of the early inflammatory phase of wound healing and can amplify inflammatory signaling, accumulating evidence indicates that they also actively contribute to the resolution of inflammation. Experimental models have shown that impaired neutrophil recruitment results in delayed or defective healing, supporting the concept that neutrophils possess essential reparative functions beyond their classical antimicrobial roles. Studies in sterile injury models further demonstrate that neutrophils directly and indirectly promote tissue restoration, not only through pathogen clearance but also by modulating the behavior of other immune cells. A critical mechanism involves the efferocytosis of apoptotic neutrophils by wound macrophages, which induces a transcriptional program associated with tissue remodeling. During apoptosis, neutrophils upregulate “eat-me” signals such as phosphatidylserine, enhancing the macrophage phagocytic capacity and driving polarization toward a reparative phenotype characterized by increased production of TGF- $\beta$  and IL-10.<sup>72,73</sup>

Consistent with these functional shifts, neutrophils undergo marked transcriptional reprogramming upon extravasation into wounded tissue. Compared with circulating neutrophils, wound-infiltrating neutrophils upregulate genes involved in leukocyte recruitment and activation, angiogenesis, and stimulation of keratinocyte and fibroblast proliferation, thereby supporting coordinated tissue repair.<sup>73</sup> Within the wound microenvironment, neutrophils also produce cytokines such as tumor necrosis TNF- $\alpha$ , which contributes to re-epithelialization and wound closure. Clearance of neutrophils by macrophages *via*  $\beta$ 2-integrin-dependent phagocytosis following removal of PAMPs and DAMPs constitutes a potent signal for macrophage-derived TGF- $\beta$ 1 release, promoting myofibroblast differentiation, wound contraction, and collagen deposition. Moreover, neutrophils facilitate further recruitment of macrophages and T cells through upregulation of monocyte chemoattractant protein-1 (MCP-1) and CXC chemokine ligand 3



(CXCL3). Finally, neutrophil-derived carbonic anhydrases modulate the wound microenvironment, thereby supporting healing under metabolically or physiologically compromised conditions.<sup>65</sup>

### 5.6. Bone

Bone fractures cause cellular damage at the defect site and a local release of cytokines, which present as a fracture hematoma.<sup>74</sup> Fracture healing is a key example of tissue repair in which neutrophils are involved.<sup>57</sup> Neutrophils are the first cells to arrive at the fracture site and recruit bone marrow mesenchymal stromal cells (BMSCs) and macrophages, with subsequent chondrogenic and osteogenic differentiation of BMSCs, regulated by macrophages.<sup>57,74</sup>

Bone fracture healing proceeds through three partially overlapping phases: an initial inflammatory phase, a reparative phase involving soft callus formation and intramembranous and endochondral ossification, and a remodeling phase in which woven bone is progressively replaced by lamellar bone, restoring the original bone architecture.<sup>65</sup> The environment associated with FH is characterized by low pH, hypoxia, high lactate levels, and elevated concentrations of inflammatory cytokines that attract neutrophils to the fracture site, which play a critical regulatory role in this process by coordinating inflammation resolution and activating downstream reparative responses.<sup>57,65</sup> Experimental evidence indicates that neutrophil depletion skews progenitor cell differentiation toward an osteogenic fate while suppressing chondrogenesis, a shift that may favor intramembranous ossification but impair diaphyseal fracture healing, where cartilaginous callus formation is required. Conversely, enhanced neutrophil recruitment induced by G-CSF improves fracture healing by increasing bone formation, strengthening biomechanical properties, and upregulating angiogenic and osteogenic factors, including VEGF, angiopoietins, bone morphogenetic protein-2 (BMP-2), and BMP-4, within the fracture callus.<sup>65</sup> In line with these findings, early neutrophil infiltration into the FH has been shown to promote fibronectin deposition, forming a provisional “emergency ECM” that facilitates stromal cell recruitment and supports effective bone repair.<sup>57,75</sup>

Several studies described a positive role of neutrophils in bone regeneration, and recent data aimed to connect the heterogeneity of neutrophil phenotypes with the different effects of neutrophils on bone regeneration, aligning with the recognition that the N1 or N2 phenotype plays an inflammatory or regenerative role.<sup>57</sup> Using *in vivo* orthotopic and ectopic models of endochondral ossification combined with *in vitro* cellular and molecular analyses, Cai *et al.*<sup>74</sup> demonstrated that moderate levels of IL-8 promote the polarization of neutrophils toward an anti-inflammatory, reparative N2 phenotype, which is essential for endochondral ossification-based bone regeneration. Following resolution of the initial inflammatory phase, cytokine concentrations within the defect microenvironment decrease to levels that favor the recruitment and polarization of late-arriving neutrophils into the N2 subtype, thereby supporting bone repair. In addition to their direct effects, N2-

polarized neutrophils contribute to macrophage reprogramming toward a reparative M2 phenotype, which subsequently regulates BMSC differentiation through growth factor secretion and suppression of inflammation. Mechanistically, under defined IL-8 concentrations, N2 neutrophils secrete stromal cell-derived factor-1 $\alpha$ , which recruits BMSCs through activation of the SDF-1/CXCR4 axis and its downstream phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway, promoting  $\beta$ -catenin-dependent cell migration.  $\beta$ -Catenin activation further induces fibronectin gene transcription, contributing to restoration of ECM components that are disrupted during the early inflammatory phase dominated by N1 neutrophils.<sup>74</sup>

Consistent with these findings, the same authors further showed in murine fracture models that IL-8 selectively recruits N2 neutrophils to the injury site and that TGF- $\beta$  contributes to the phenotypic conversion from N1 to N2 neutrophils.<sup>74</sup> A complementary *in vitro* study described the comparison of angiogenic and osteogenic gene expression in a co-culture of human neutrophils, HOBs, and HUVECs with a co-culture of HOBs and HUVECs. Co-culture of human neutrophils with HOBs and HUVECs resulted in increased expression of angiogenic and osteogenic markers, including VEGF-A, CD34, FGF-2, alkaline phosphatase, type I collagen, osteopontin, and osteocalcin, compared with osteoblast–endothelial co-cultures alone, supporting a direct pro-regenerative role for neutrophils.<sup>57</sup>

Finally, temporal changes in neutrophil-derived cytokine expression reflect a dynamic shift in the neutrophil phenotype during fracture healing. TNF- $\alpha$ , predominantly produced by N1 neutrophils, is associated with early neutrophil recruitment during the inflammatory phase, whereas IL-8 expression increases at later stages and is mainly derived from N2 neutrophils. This cytokine switch serves as an indicator of the transition from an inflammatory to a regenerative neutrophil population. Although current *in vivo* evidence remains limited, an increasing number of studies support the presence and functional relevance of N1 and N2 neutrophil subsets during bone healing.<sup>57</sup>

### 5.7. Muscle

Skeletal muscle, also referred to as striated muscle, is the most abundant tissue in the human body and plays a fundamental role in locomotion and metabolic homeostasis. Skeletal muscle injury commonly results from trauma, ischemia–reperfusion, burns, or strenuous physical activity, yet effective therapeutic strategies remain limited. Increasing attention has been directed toward the complex crosstalk between neutrophils and skeletal muscle in both acute injury and chronic disease settings.<sup>58</sup>

Following muscle injury, neutrophils remove necrotic tissue fragments and invading microorganisms, thereby creating a permissive environment for subsequent regeneration. This process is supported by the release of neutrophil-derived cytokines, proteolytic enzymes, and NETs. Skeletal muscle repair is a multistep process involving inflammation, myofiber regener-



ation, angiogenesis, and ECM remodeling. Infiltrating neutrophils promote angiogenesis through mitogen-activated protein kinase -dependent release of VEGF and MMP-9 and accelerate wound healing *via* secretion of secretory leukocyte protease inhibitor following activation of NF- $\kappa$ B signaling.<sup>58</sup> Neutrophils also support muscle regeneration by activating satellite cells through STAT-dependent signaling pathways. During later stages of repair, neutrophils undergo apoptosis or egress from the tissue, and their clearance by macrophages initiates a feedback repair program characterized by the release of anti-inflammatory and pro-reparative mediators, including TGF- $\beta$  and IL-10. These factors promote ECM remodeling and restoration of normal muscle contractile function.<sup>58</sup> In this context, neutrophil-derived TGF- $\beta$  and BMP signaling influences stromal cell fate decisions. TGF- $\beta$  regulates scleraxis expression in skeletal muscle fibroblasts, promoting their proliferation and collagen type I synthesis, whereas BMP signaling drives mesenchymal stromal cells toward an osteogenic lineage, processes that must be tightly controlled to ensure functional tissue repair.<sup>58</sup>

Furthermore, a research study demonstrated that TNF- $\alpha$  selectively activates the chemokine ligand (CCL)20-chemokine receptor (CCR)6 axis, recruiting a subset of VEGF-A-expressing neutrophils with proangiogenic properties to areas affected by ischemic injury, thereby facilitating revascularization. Beyond debris clearance and satellite cell activation, neutrophils also act as a source of interleukin-6 (IL-6), which directly promotes muscle regeneration by activating STAT3. STAT3 functions downstream of the IL-6 receptor and plays a central role in regulating satellite cell expansion and skeletal muscle repair.<sup>20</sup>

## 6. Neutrophils in biomaterial-based tissue repair

### 6.1. Neutrophil response to biomaterials

Any implanted biomaterial or biomedical device will be recognized by the host immune system as foreign and an immune response known as the Foreign Body Response (FBR) will be initiated.<sup>76</sup> The FBR can be described in five stages that occur sequentially in time. The first phase is governed by the adsorption of proteins onto the surface of the biomaterial creating a provisional matrix that will be of key importance for the following immune cell interactions. The second phase is the acute inflammatory response that, if not properly resolved, leads to the third phase, the chronic inflammatory response. The fourth phase is dominated by the formation of foreign body giant cells at the surface of the material, and this response is finalized by the formation of a fibrous capsule surrounding the implant.<sup>5,77-79</sup> We will herein provide an overview of the role of neutrophils in this response.

Circulating PMN leukocytes, mainly neutrophils, are promptly recruited to the implant site and are the first immune cells to interact with the biomaterial and they will shape the early inflammatory microenvironment.<sup>1</sup> The process of neutrophil migration to the injured site is considered to

have three phases: (i) neutrophil forward migration; (ii) amplification of neutrophil recruitment and (iii) neutrophil reverse migration.<sup>80</sup>

The implantation of a biomaterial causes injury to the surrounding cells and these damaged cells will secrete DAMPs, IL-8 and leukotriene B4 (LTB4) causing neutrophil recruitment from the circulation.<sup>17</sup> The segmented nucleus morphology enables a rapid neutrophil migration from blood vessels and through the gaps between cells and the ECM.<sup>81</sup> The activation of the complement system has also an important role in neutrophil recruitment since many biomaterials trigger the activation of this pathway, leading to the activated form of complement proteins that act as potent neutrophil chemoattractants.<sup>82</sup> Neutrophils may persist in the biomaterial implantation site for longer periods of time than 24 hours or less originally thought, having a key role not only in the acute inflammatory response, but also in the subsequent macrophage recruitment and polarization, activation of fibroblasts and fibrous encapsulation.<sup>83,84</sup>

Neutrophil response to implanted biomaterials will differ according to the material size. If sufficiently small the biomaterial will be phagocytized; alternatively, the neutrophil will create a degradative microenvironment through the secretion of robust amounts of ROS and proteolytic enzymes in an attempt to degrade the implant.<sup>85</sup> At the surface of the biomaterial, neutrophils form NETs that will contribute to the formation of a fibrotic matrix, hindering biomaterial-tissue integration.<sup>86,87</sup> Neutrophils are active secretory cells; they will produce several pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, which will amplify the inflammatory response. Additionally, neutrophils will secrete IL-8 that will further recruit PMNs and will secrete MCP-1 and MIP-1 $\beta$  that will act as chemoattractants to monocytes and macrophages, as well as lymphocytes and immature DCs.<sup>88-90</sup> The microenvironment created by the secretion of these cytokines influences macrophage polarization. A highly pro-inflammatory environment may promote a classically activated (M1) macrophage phenotype, associated with chronic inflammation and fibrotic encapsulation. On the other hand, a more regulated neutrophil response may support a transition toward pro-healing (M2-like) macrophages.<sup>91</sup> Additionally, the number of recruited macrophages is correlated with the initial number of neutrophils, highlighting the importance of these cells in the acute response.<sup>92</sup>

Besides having an important role in the onset of the inflammatory response to biomaterials, neutrophils are also important players of the resolution of this response.<sup>92</sup> Neutrophil apoptosis is important for macrophage polarization towards an M2 anti-inflammatory phenotype and thus for the resolution phase of inflammation.<sup>93</sup> Additionally, neutrophils may leave the inflammatory site in a process named reverse migration which is also considered as a mechanism to resolve inflammation.<sup>80</sup> Finally, near the end of an acute inflammatory response neutrophils can shift the synthesis of LTB4 into Lipoxin A4, a molecule that belongs to the SPM family that acts towards the resolution of inflammation.<sup>94</sup>



Taking into consideration the key role of neutrophils in both the onset and resolution of the inflammatory response to biomaterials, we will now explore their potential in the development of immunomodulatory biomaterials for tissue repair/regeneration.

## 6.2. Biomaterials for revascularization and immunomodulation through neutrophil modulation

The growing knowledge that the immune system is crucial for tissue homeostasis has led to the interest in regenerative medicine and immunomodulatory therapies for transforming inflammation into tissue repair.<sup>4,95</sup> The current emphasis is on the development of biomaterials with immunomodulatory properties that can modulate selective immune responses towards healing and regeneration.<sup>4,8</sup>

Since neutrophil infiltration typically peaks within the first 24–48 hours post-implantation, the focus of tissue engineering strategies has been on limiting the recruitment of neutrophils.<sup>17</sup> Experimental evidence supports the concept that modulation of early neutrophil responses can significantly improve healing outcomes. An anti-inflammatory hydrogel composed of heparin and star-shaped polyethylene glycol (PEG) was shown to sequester pro-inflammatory chemokines—including IL-8, MCP-1, MIP-1 $\alpha$ , and MIP-1 $\beta$ —from diabetic wound fluid, thereby reducing neutrophil and monocyte migration. *In vivo*, this strategy attenuated inflammation, enhanced granulation tissue formation and vascularization within 10 days, and promoted superior wound closure compared with the commercially available dressing Promogran.<sup>4</sup> Similarly, synthetic polymers such as poly(lactic acid), poly(lactic-co-glycolic acid) (PLGA), and poly(vinyl alcohol) have been reported to enhance the biocompatibility by minimizing protein adsorption and limiting neutrophil recruitment.<sup>18</sup> Moreover, encapsulation of aspirin-triggered resolvin D1 (AT-RvD1) within a PLGA scaffold reduced the neutrophil presence and migration in a murine dorsal skinfold model. This approach also decreased the neutrophil-to-monocyte/macrophage ratio, indicating the attenuation of inflammation and improved wound healing, while supporting vascular remodeling processes.<sup>96</sup>

The last decade has seen remarkable progress in the biomaterial-based immunomodulation of neutrophils, since their outstanding plasticity and potential to promote revascularization and tissue remodeling were discovered (Table 1). The activation and functional profile of neutrophils are strongly modulated by the physicochemical properties of the biomaterial (origin, topography, hydrophobicity, chemical composition) and the surrounding microenvironment, thereby shaping subsequent immune responses.<sup>9,18,30</sup>

Advancements in this field have been constrained by the limited lifespan of neutrophils in circulation and the intricate mechanisms governing their biological regulation. Early research has predominantly focused on neutrophil behavior at the biomaterial interface, emphasizing phenomena such as apoptosis and NETosis, particularly in relation to the foreign body response to implanted devices.<sup>10</sup>

We will herein explore three main strategies employed to modulate neutrophil responses: (i) promoting neutrophil apoptosis followed by macrophage-mediated efferocytosis to facilitate the resolution of inflammation; (ii) controlling the production and activity of ROS and NETs to limit tissue damage; and (iii) modulating neutrophil polarization to favor pro-resolving and regenerative phenotypes.

### 6.2.1. Promoting neutrophil apoptosis and efferocytosis.

The clearance of neutrophils from the injury site can occur by apoptosis and subsequent engulfment by macrophages (efferocytosis), which leads to a phenotypic change to an anti-inflammatory profile in macrophages. This shift contributes to the restoration of homeostasis and promotes regeneration. Therefore, inducing neutrophil apoptosis in a timely manner arises as a strategy to induce transiently heightened inflammation primers for regeneration. To investigate this, a phenylboronic acid (PBA)-based polymeric hydrogel loaded with fMPLP/SiO<sub>2</sub>-FasL complexes (Gel@fMPLP/SiO<sub>2</sub>-FasL) was developed to achieve precise temporal and spatial regulation of neutrophil recruitment and apoptosis to modulate inflammation and enhance tissue regeneration. The PBA-based polymeric hydrogels were formed using chitosan modified with 4-formylphenylboronic acid and subsequently loaded with the chemoattractant formyl-Met-Leu-Phe (fMPLP) and Fas ligand-conjugated silica nanoparticles (SiO<sub>2</sub>-FasL). Upon implantation, the hydrogel enabled an initial burst release of fMPLP, promoting rapid neutrophil recruitment and transient amplification of the inflammatory response. Subsequently, acidification of the microenvironment by activated neutrophils triggered hydrogel degradation, exposing SiO<sub>2</sub>-FasL and inducing neutrophil apoptosis *via* FasL–Fas signaling, thereby promoting timely resolution of inflammation. Apoptotic neutrophils were then cleared by macrophages, leading to efferocytosis-mediated activation of anti-inflammatory signaling pathways and macrophage polarization toward a pro-regenerative phenotype. The biomaterial was evaluated *in vivo* using murine models of critical-sized calvarial bone defects and diabetic cutaneous wounds. In both models, Gel@fMPLP/SiO<sub>2</sub>-FasL enhanced the macrophage phenotypic transition, increased anti-inflammatory cytokine levels, and reprogrammed the local microenvironment toward regeneration. In the calvarial defect model, this immunomodulatory cascade promoted the recruitment of stem cells from bone sutures, supporting their differentiation and intramembranous ossification, ultimately restoring the bone morphology and structure. In the diabetic wound model, the treatment facilitated stem cell recruitment, angiogenesis, and re-epithelialization, leading to improved wound healing outcomes.<sup>97</sup> Li *et al.*<sup>98</sup> evaluated the role of the osteoimmune microenvironment in bone repair following the implantation of various biomaterials in murine tibia. The study employed single-cell RNA sequencing to characterize cellular populations surrounding implant materials with distinct osteogenic properties, to elucidate the immune microenvironment contributing to biomaterial-mediated bone regeneration. The researchers investigated sandblasted, large grit and acid-etched titanium (SLA), strontium-incorporated SLA, polished titanium,



**Table 1** Biomaterial-induced neutrophil immunomodulation

Biomaterial	<i>In vitro</i> model	<i>In vivo</i> model	Effects on neutrophil immunomodulation/ tissue repair	Ref.
Chitosan-based hydrogel loaded with fMLP and FasL-conjugated silica nanoparticles (SiO <sub>2</sub> -FasL) (Gel@fMLP/SiO <sub>2</sub> -FasL)	Primary murine bone marrow-derived neutrophils Bone marrow-derived macrophages	Mice (critical-sized calvarial bone defect and diabetic cutaneous wound model)	Enhanced early neutrophil recruitment (fMLP-mediated)  FasL-induced apoptosis of activated neutrophils, promoting macrophage polarization to M2 and transiently heightened inflammatory response that improved tissue regeneration.	95
Titanium implants (modified surfaces)	Not applicable	Mice (tibia implantation)	Strontium ion modification of Ti recruited mature neutrophils and highly differentiated macrophages, achieving functional osseointegration	96
Medical stainless steel (control)			Neutrophils may improve bone formation by enhancing the recruitment of BMSCs	
Microchanneled 3D-printed polycaprolactone (PCL) scaffold	Not applicable	Mice (critical-sized calvarial bone defect)	Reduced NET formation Reduced foreign body reaction Enhanced angiogenesis	97
Silk fibroin hydrogel loaded with metformin-loaded mesoporous silica microspheres and silver nanoparticles	Murine bone marrow neutrophils	Mice (diabetic wound model)	<i>In vitro</i> results: Reduced NET formation Reduced release of NET-associated components (neutrophil elastase and myeloperoxidase) <i>In vivo</i> results: Reduced NET deposition Decreased pro-inflammatory neutrophil mediators	98
Titanium surfaces (distinct topographies and hydrophilicity)	Primary murine neutrophils	Not applicable	Reduced pro-inflammatory cytokine and enzyme production and decreased NET formation on hydrophilic/rough surfaces Inhibition of NETosis enhanced anti-inflammatory macrophage polarization	100
Polydimethylsiloxane substrates (different rigidity)	Murine neutrophils	Not applicable	Increased NET formation and pro-inflammatory cytokine/chemokine secretion on higher stiffness substrates	99
Isodecyl acrylate copolymers functionalized with carboxyl and amino groups	HL-60 cells and murine bone marrow-derived neutrophils	Not applicable	Reduced NETosis and lower inflammatory profile on carboxyl-functionalized films	101
Electrospun templates: polydioxanone (PDO), collagen (COL), and blended PDO-COL fibers with different diameters	Human neutrophils	Mice (subcutaneous implantation)	<i>In vitro</i> results: Reduced NETosis on larger-diameter PDO fibers <i>In vivo</i> results: Reduced NET deposition on large-diameter PDO templates correlating with improved tissue integration	102
Selection of hard, soft, natural vs. synthetic polymeric biomaterials (THA, THA-collagen, collagen, GelMA, polyvinyl alcohol, tissue culture plastic, and PCL)	Primary human neutrophils	Not applicable	Higher neutrophil survival, reduced myeloperoxidase and elastase release, decreased cytokine secretion, and lower NET formation on naturally-derived polymers	90
Bioengineered vascular networks (assembled vs. unassembled vascular grafts)	Not applicable	Mice (subcutaneous implantation)	Implantation of unassembled vascular networks led to successful anastomoses and vessel patency Non-inflammatory phenotype that is consistent with the N2 phenotype was displayed on unassembled grafts	28
Mesoporous bioactive glass scaffold combined with hyaluronic acid methacryloyl hydrogel loaded with TGF- $\beta$ 1 adenovirus (Ad@H/M)	Primary mouse neutrophils	Mice (calvarial defect model)  Rabbit (radial defect model)	<i>In vitro</i> results: TGF- $\beta$ 1 promoted neutrophil polarization to N2 and subsequently induced macrophage polarization toward M2 <i>In vivo</i> results: In tissues implanted with Ad@H/M, the N2 phenotype was predominant over N1	103
PCL nanofibrous membrane functionalized with LAPONITE® (PCL/LAP)	Periodontal ligament cells (PDLCs)	Mice (periodontal defect model)	<i>In vitro</i> results: Downregulation of classical pro-inflammatory genes and upregulation of anti-inflammatory and pro-remodeling genes in neutrophils (N2 polarization)	104
Gelatin methacrylate (GelMA) hydrogels (distinct stiffness values)	Murine bone marrow-derived neutrophils	Not applicable	Neutrophil polarization toward the N2 phenotype promoted on stiffer matrices	105



and medical stainless steel to clarify the influence of the osteoimmune microenvironment and cellular heterogeneity on bone regeneration. Findings suggest that neutrophils may positively affect bone formation, potentially by recruiting bone marrow stromal cells through the chemokine ligand CXCL12/CXCR3 axis. Neutrophil-depletion mouse models were used to further explore the function of neutrophils in osseointegration, supporting the hypothesis that neutrophils enhance bone formation by facilitating the recruitment of BMSCs via CXCL12/CXCR3 signaling.

**6.2.2. Manipulating ros and net formation.** Several experimental studies have demonstrated that the physicochemical properties of biomaterials—including architecture, composition, stiffness, surface topography, and functional groups—critically regulate neutrophil activation and NET formation, thereby influencing tissue repair outcomes. Using 3D printing, Won *et al.*<sup>99</sup> fabricated a hierarchical polycaprolactone (PCL) scaffold incorporating microchannels. *In vivo* evaluation with mice demonstrated that, compared with chemically identical PCL scaffolds lacking microchannels, the microchannel-containing scaffolds significantly reduced NET formation, promoted inflammation resolution, and enhanced angiogenesis, highlighting the impact of structural design on neutrophil behavior. Similarly, Mei *et al.*<sup>100</sup> developed a multifunctional methacrylated silk fibroin hydrogel incorporating silver nanoparticles and metformin-loaded mesoporous silica nanoparticles for diabetic wound healing. *In vitro* assays using isolated murine bone marrow neutrophils showed that conditioned media from the composite hydrogel significantly reduced NET formation and NET-associated components (neutrophil elastase and myeloperoxidase) compared with controls. Subsequent *in vivo* studies in diabetic mouse models confirmed reduced NET deposition, decreased pro-inflammatory neutrophil mediators, enhanced fibroblast migration, increased angiogenesis, and improved collagen deposition, collectively promoting wound healing.

Surface properties have also been shown to modulate neutrophil responses. Abaricia *et al.*<sup>101</sup> investigated primary murine neutrophils cultured *in vitro* on titanium surfaces with distinct topographies and hydrophilicity. Neutrophils on rough, hydrophilic titanium exhibited reduced pro-inflammatory cytokine and enzyme production and decreased NET formation compared with smooth or rough hydrophobic titanium surfaces. Furthermore, inhibition of NETosis enhanced anti-inflammatory macrophage polarization, demonstrating that neutrophil-derived signals influence downstream immune responses.<sup>102</sup> Material stiffness also plays a role in modulating immune responses. Murine neutrophils were tested on *in vitro* assays using polydimethylsiloxane substrates of varying rigidity. The results showed that increased stiffness enhanced NET formation and pro-inflammatory cytokine secretion through integrin/focal adhesion kinase signaling pathways.

Chemical functionalization further determines neutrophil behavior. Fong and Wells<sup>103</sup> evaluated isodecyl acrylate copolymers functionalized with methacrylic acid (MAA, carboxylic groups), methyl methacrylate, or hexamethylenediamine

(HMD, amine groups). *In vitro* experiments using phorbol myristate acetate-activated HL-60 cells and murine bone marrow-derived neutrophils demonstrated increased NET accumulation and pro-inflammatory cytokine production on HMD films, whereas MAA films were associated with reduced NETosis and a lower inflammatory profile.

Template architecture has also been implicated in regulating NETosis. Fetz *et al.*<sup>104</sup> fabricated electrospun polydioxanone (PDO), collagen type I (COL), and blended PDO-COL scaffolds with small (0.25–0.35  $\mu\text{m}$ ) or large (1.0–2.0  $\mu\text{m}$ ) fiber diameters. *In vitro* assays with human neutrophils demonstrated reduced NETosis on larger-diameter PDO fibers, while collagen incorporation attenuated NET formation irrespective of the fiber size. *In vivo* validation using a rat subcutaneous implantation model revealed reduced NET deposition on large-diameter PDO templates at 24 hours, correlating with improved tissue integration at 7 days. Conversely, small-diameter PDO scaffolds exhibited extensive NET coating and subsequent capsule-like tissue formation, suggesting that early NETosis may precondition the innate immune response and influence regenerative outcomes.

Wesdorp *et al.*<sup>92</sup> systematically compared primary human neutrophil responses *in vitro* with naturally derived and synthetic biomaterials, including tyramine-functionalized hyaluronic acid (THA), THA-collagen composites, collagen type I, gelatin methacryloyl (GelMA), polyvinyl alcohol, tissue culture plastic, and PCL. Neutrophils cultured on naturally derived materials displayed higher survival, reduced myeloperoxidase and neutrophil elastase release, decreased cytokine secretion, and lower NET formation compared with synthetic substrates, underscoring the importance of material composition in shaping neutrophil-mediated immune responses. Collectively, these findings emphasize that biomaterial design parameters critically influence neutrophil activation and NETosis, thereby modulating inflammation resolution and regenerative success.

**6.2.3. Modulating neutrophil polarization.** Targeting neutrophil polarization has emerged as a promising immunomodulatory strategy in tissue engineering, complementing the well-established focus on macrophage polarization. Given their role as first responders and their capacity to initiate angiogenesis, modulating neutrophil phenotypes at early stages of healing may shape subsequent immune cascades toward a pro-regenerative microenvironment.<sup>17</sup> Several experimental studies have explored this concept using biomaterial-based approaches in both *in vitro* and *in vivo* models.

Lin *et al.*<sup>30</sup> recently assessed the capacity of neutrophils to facilitate anastomosis formation with bioengineered microvessels in 3D hydrogels through implantation into mice. Their study demonstrated that implantation of unassembled vascular networks led to successful anastomoses and vessel patency, whereas pre-assembled, mature vascular networks did not establish connections with host tissue. It has been observed that hypoxic transplanted tissues recruit neutrophils, which deliver proangiogenic MMP-9, through a VEGF-dependent mechanism. Depletion of host neutrophils resulted in inhibition of vascularization within the unassembled networks,



indicating that neutrophil polarization and active involvement in producing angiogenic factors are essential for anastomosis formation.

Shi *et al.*<sup>105</sup> developed a genetically engineered composite scaffold consisting of a mesoporous bioactive glass framework filled with a hyaluronic acid methacryloyl hydrogel loaded with a TGF- $\beta$ 1 adenovirus (Ad@H/M). *In vitro* assays with primary mouse neutrophils demonstrated that TGF- $\beta$ 1 delivery promoted neutrophil polarization toward the N2 phenotype and subsequently induced macrophage polarization toward the M2 phenotype through a coordinated “relay” mechanism involving Janus kinase (JAK)/STAT3 and PI3K/AKT/NF- $\kappa$ B signaling pathways. *In vivo* validation in murine calvarial defect and rabbit radial defect models confirmed enhanced mesenchymal stem cell recruitment, osteoblast and endothelial cell differentiation, and improved bone regeneration, including morphological remodeling.

The matrix composition and architecture of the biomaterial also regulate the neutrophil phenotype. Xu *et al.*<sup>106</sup> prepared electrospun PCL/LAPONITE® (LAP) nanofibrous membranes and showed that conditioned media from cocultures with periodontal ligament cells (PDLs) stimulated the proliferation and osteogenesis of PDLs and downregulated classical pro-inflammatory genes and upregulated anti-inflammatory and pro-remodeling genes in neutrophils, suggesting N2 polarization. PCL/LAP implantation *in vivo* had a synergistic effect on rat periodontal tissue regeneration. Similarly, substrate stiffness has been shown to influence neutrophil behavior. Using 3D GelMA hydrogels with different stiffness values, Jiang *et al.*<sup>107</sup> cultured murine bone marrow-derived neutrophils and demonstrated that stiffer matrices promoted N2 polarization, characterized by reduced adhesion molecule expression, decreased ROS production, and increased anti-inflammatory cytokine secretion. RNA sequencing and inhibition assays confirmed that a stiffer matrix promotes the shift to the N2 phenotype and this effect was mediated *via* activation of the JAK1/STAT3 signaling pathway. Gao *et al.*<sup>29</sup> also engineered a bioactive microporous GelMA hydrogel scaffold incorporating conditioned media derived from N2-polarized neutrophils. *In vitro* assays with HUVECs demonstrated enhanced endothelial migration, sprouting, and tubular network formation. *In vivo* studies in male and nude mice confirmed that N2-conditioned GelMA scaffolds reduced inflammatory cell recruitment, maintained endothelial cell survival, promoted vascular anastomosis and maturation, and accelerated revascularization in ischemic tissues. These effects were associated with upregulation of anti-inflammatory and vascular maturation genes, highlighting the therapeutic potential of N2-targeted immunomodulatory hydrogels.

Collectively, these studies demonstrate that biomaterial-driven modulation of neutrophil polarization—particularly toward the N2 phenotype—represents a powerful strategy to orchestrate macrophage responses, enhance angiogenesis, and promote functional tissue regeneration.

## 7. Conclusions and future directions

The classical view of neutrophils as short-lived, terminally differentiated, and predominantly pro-inflammatory cells has been fundamentally revised over the last decade. Accumulating evidence demonstrates that neutrophils are highly plastic immune regulators capable of orchestrating inflammation resolution, angiogenesis, ECM remodeling, stem cell recruitment, and tissue regeneration across multiple organ systems. These cells are now recognized as dynamic coordinators of regenerative microenvironments, capable of exerting both pro- and anti-inflammatory effects.<sup>2,49</sup>

In the context of biomaterial-based tissue engineering, it is increasingly evident that early neutrophil–material interactions critically influence the foreign body response and regenerative outcomes. Material properties—including stiffness, surface chemistry, architecture, and degradability—modulate neutrophil apoptosis, NET formation, ROS production, and polarization states. Natural polymers generally generate bioresorbable degradation products but may degrade rapidly and trigger immune activation, whereas synthetic polymers provide greater mechanical stability yet may lack intrinsic biocompatibility. Hybrid systems combining natural and synthetic components offer a rational approach to tailor degradation profiles and mechanical properties while maintaining the immune compatibility. Evidence discussed herein suggests that hydrophobic materials, substrates with lower stiffness, and natural polymers, when compared to more rigid or purely synthetic alternatives, tend to promote an anti-inflammatory neutrophil phenotype. In this context, fine-tuning the physical and chemical properties of biomaterials emerges as a promising strategy to direct neutrophil behavior and enhance biomaterial–tissue integration, ultimately improving tissue integration and vascularization.<sup>29,108</sup>

Despite recent technological advances, early host–material interactions and neutrophil-mediated mechanisms driving vascularization remain incompletely understood. More physiologically relevant *in vitro* models—particularly 3D systems that extend neutrophil viability—together with advanced *in vivo* imaging approaches are needed to better capture early inflammatory dynamics and intercellular crosstalk after implantation. Although single-cell and spatial transcriptomic analyses have uncovered neutrophil heterogeneity, key questions persist regarding the temporal regulation of polarization, metabolic adaptation within biomaterial niches, and the molecular checkpoints controlling the transition from inflammatory amplification to regenerative signaling.<sup>57,109</sup>

Future efforts should focus on designing biomaterials that temporally program neutrophil responses rather than merely suppress early inflammation. Smart scaffolds with controlled release mechanisms and responsive degradation profiles may enable sequential regulation of recruitment, activation, and resolution, thereby mimicking physiological wound healing and improving outcomes in chronic inflammatory conditions. Translational progress will further depend on integrating advanced experimental platforms with computational model-



ing to guide predictive biomaterial design. Ultimately, incorporating patient-specific immune profiling into biomaterial development will be essential, as neutrophil function is strongly influenced by individual clinical variables. Harnessing neutrophil plasticity represents a promising frontier in regenerative medicine, with the potential to transform biomaterial integration from passive biocompatibility to active immune-guided regeneration.

## Conflicts of interest

There are no conflicts to declare.

## Abbreviations

3D	Three-dimensional
AA	Arachidonic acid
AKT	Protein kinase B
ALI	Acute lung injury
AMI	Acute myocardial infarction
ATP	Adenosine triphosphate
BMP	Bone morphogenetic proteins
BMSCs	Bone marrow mesenchymal stromal cells
CCL	Chemokine ligand
CCR	Chemokine receptor
CD	Cluster of differentiation
CNS	Central nervous system
COL	Collagen type I
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
Cyr61	Cysteine-rich protein 61
DAMPs	Damage-associated molecular patterns
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EPA	Eicosapentaenoic acid
FBR	Foreign body response
FGF	Fibroblast growth factor
fMLP	Formyl-Met-Leu-Phe
G-CSF	Granulocyte colony-stimulating factor
G-MDSC	Granulocytic myeloid derived suppressor cells
GelMA	Gelatin methacryloyl
GPCRs	G-protein-coupled receptors
HDNs	High-density neutrophils
HGF	Hepatocyte growth factor
HMGB1	High mobility group protein B1
HMD	Hexamethylenediamine
HOBs	Human osteoblasts
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule 1
IFN- $\gamma$	Interferon- $\gamma$
IL	Interleukin
JAK	Janus kinase
LAP	LAPONITE®

LDNs	Low-density neutrophils
LIFR	Leukemia inhibitory factor receptor
LPC	Lysophosphatidylcholine
LPS	Lipopolysaccharides
LTB4	Leukotriene B4
MAA	Methacrylic acid
Mac-1	Macrophage antigen 1
MCP-1	Monocyte chemoattractant protein-1
MIP	Macrophage inflammatory protein
MMP-8	Matrix metalloproteinase-8
MMP-9	Matrix metalloproteinase-9
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NADPH	Nicotinamide adenine dinucleotide phosphate
NDNs	Normal-density neutrophils
NETs	Neutrophil extracellular traps
NF- $\kappa$ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
Nox2	NADPH oxidase 2
n-3 DPA	n-3 docosapentaenoic acid
PAMPs	Pathogen-associated molecular patterns
PANs	Pro-angiogenic neutrophils
PBA	Phenylboronic acid
PCL	Polycaprolactone
PDLCS	Periodontal ligament cells
PDO	Polydioxanone
PI3K	Phosphatidylinositol 3-kinase
PLGA	Poly(lactic-co-glycolic acid)
PMN	Polymorphonuclear
PNS	Peripheral nervous system
PS	Phosphatidylserine
RNA	Ribonucleic acid
ROS	Reactive oxygen species
S1P	Sphingosine 1-phosphate
SCI	Spinal cord injury
SiO <sub>2</sub> -FasL	Fas ligand-conjugated silica nanoparticles
SLA	Acid-etched titanium
SPMs	Specialized pro-resolving mediators
STAT3	Signal transducer and activator of transcription 3
TANs	Tumor-associated neutrophils
TGF- $\beta$	Transforming growth factor- $\beta$
THA	Tyramine-functionalized hyaluronic acid
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
VCAM-1	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor
VEGF-A	Vascular endothelial growth factor A
$\alpha$ -Ly6G	Lymphocyte antigen 6 complex locus G6D.

## Data availability

This article is a review of previously published work and does not report any new experimental data. Therefore, no new data were generated or analyzed in support of this research.



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