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Review article

Promoting tissue regeneration by modulating the immune system

Ziad Julier [a,1], Anthony J. Park [a,1], Priscilla S. Briquez [b], Mikaël M. Martino [a,*]

[a] European Molecular Biology Laboratory Australia, Australian Regenerative Medicine Institute, Monash University, Victoria 3800, Australia
[b] Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

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ABSTRACT

The immune system plays a central role in tissue repair and regeneration. Indeed, the immune response to tissue injury is crucial in determining the speed and the outcome of the healing process, including the extent of scarring and the restoration of organ function. Therefore, controlling immune components via biomaterials and drug delivery systems is becoming an attractive approach in regenerative medicine, since therapies based on stem cells and growth factors have not yet proven to be broadly effective in the clinic. To integrate the immune system into regenerative strategies, one of the first challenges is to understand the precise functions of the different immune components during the tissue healing process. While remarkable progress has been made, the immune mechanisms involved are still elusive, and there is indication for both negative and positive roles depending on the tissue type or organ and life stage. It is well recognized that the innate immune response comprising danger signals, neutrophils and macrophages modulates tissue healing. In addition, it is becoming evident that the adaptive immune response, in particular T cell subset activities, plays a critical role. In this review, we first present an overview of the basic immune mechanisms involved in tissue repair and regeneration. Then, we highlight various approaches based on biomaterials and drug delivery systems that aim at modulating these mechanisms to limit fibrosis and promote regeneration. We propose that the next generation of regenerative therapies may evolve from typical biomaterial-, stem cell-, or growth factor-centric approaches to an immune-centric approach.

Statement of Significance

Most regenerative strategies have not yet proven to be safe or reasonably efficient in the clinic. In addition to stem cells and growth factors, the immune system plays a crucial role in the tissue healing process. Here, we propose that controlling the immune-mediated mechanisms of tissue repair and regeneration may support existing regenerative strategies or could be an alternative to using stem cells and growth factors. The first part of this review we highlight key immune mechanisms involved in the tissue healing process and marks them as potential target for designing regenerative strategies. In the second part, we discuss various approaches using biomaterials and drug delivery systems that aim at modulating the components of the immune system to promote tissue regeneration.

Contents

* Corresponding author at: Australian Regenerative Medicine Institute, 15 Innovation Walk, Building 75, Level 1, Martino lab, Victoria 3800, Australia.
E-mail address: mikael.martino@monash.edu (M.M. Martino).
[1] These authors contributed equally.

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1. Introduction

While remarkable progress has been achieved in understanding the cellular and molecular mechanisms of tissue repair and regeneration, it remains unexplained why mammals have a tendency for imperfect healing and scarring rather than regeneration. There is ample evidence in different model organisms indicating that the immune system is crucial to determine the quality of the repair response, including the extent of scarring, and the restoration of organ structure and function. A widespread idea derived from findings in diverse species is that the loss of regenerative capacity is linked to the evolution of immune competence (Fig. 1). Still, there are many situations where the immune response to tissue injury promotes tissue healing. Indeed, the relationship between tissue healing and the immune response is very complex, since there are both negative and positive roles, depending on the tissue, organ and life stage (embryonic, neonatal or adult) [1]. The type of immune response, its duration and the cells involved can drastically change the outcome of the tissue healing process from incomplete healing and repair (i.e. scarring or fibrosis) to complete restoration (i.e. regeneration).

In regenerative medicine, strategies based on stem cells and growth factors have not yet proven broadly effective in the clinic. Here, we propose that immune-mediated mechanisms of tissue repair and regeneration may support existing regenerative strategies or could be an alternative to using stem cells and growth factors. In the first part of this review, we present key immune mechanisms involved in the tissue healing process, in order to highlight potential targets. In the second part, we discuss various approaches using biomaterials and drug delivery systems that aim at modulating the components of the immune system to promote tissue repair and regeneration.

2. The main actors of the immune response following tissue injury

An immune response almost always follows tissue damage and this response is usually resolved within days to weeks after an injury. The first phase of the immune response involves components of the innate immune system, which provide instant defense against potential pathogens invading the damaged tissue. However, even in the absence of pathogens, the immune response initially triggered by danger signals released from damaged tissues produces a so-called sterile inflammation [2,3]. In many if not all tissues, the innate immune response strongly modulates the healing process. For instance, macrophages and their various phenotypes play a predominant role in the restoration of tissue homeostasis by clearing away cellular debris, remodeling the extracellular matrix (ECM), and synthesizing multiple cytokines and growth factors. The innate immune response is then followed by the activation of the adaptive immune system. Although this was originally thought of as a secondary actor in the tissue healing process, the adaptive immune response to tissue injury most likely plays a critical role during tissue repair and regeneration, in particular the activity of T cells. While a large research effort has focused on how transplanted mesenchymal stem cells (MSCs) modulate T cell activities and immune tolerance [4,5], our understanding of how T cells modulate tissue-resident stem cells and the tissue healing process is just beginning. In the next sections, we review the roles and importance of the main actors that shape the immune response following tissue injury.

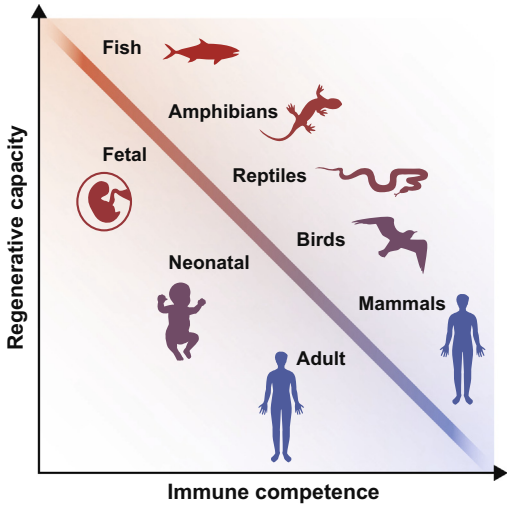

Fig. 1. Apparent inverse relationship between regenerative and immune capacities during evolution or development. Lower vertebrates such as fishes and amphibians have the ability to completely regenerate many of their tissues. In mammals, regenerative capacities depend on the developmental stage (i.e. fetal, neonatal, and adult). Immune competences have increased during evolution and also increase with life stage in mammals.

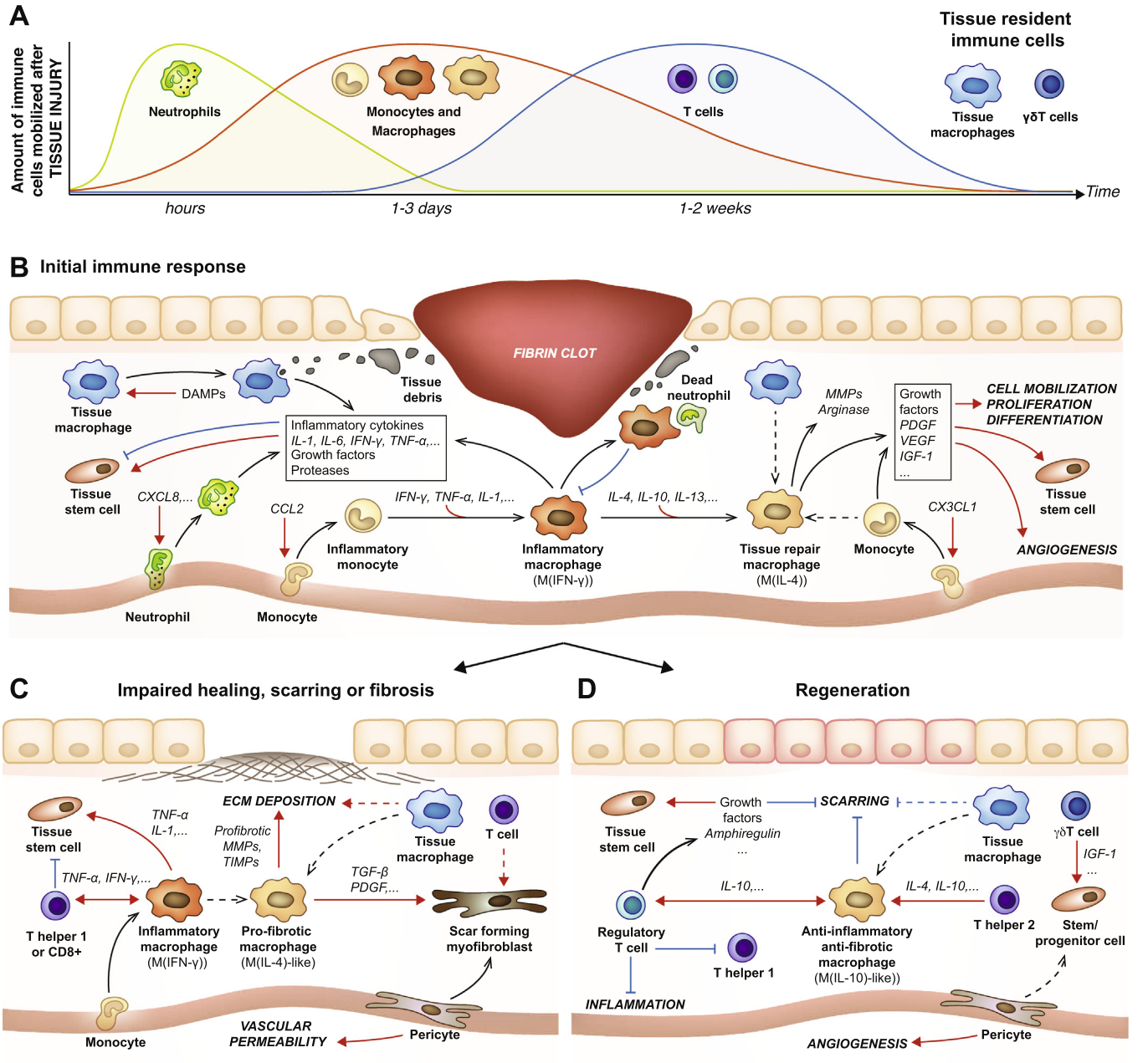

Fig. 2. The main actors of the immune response following tissue injury. (A) Kinetic of immune cell mobilization after tissue injury. Tissue resident cells including tissue-resident macrophages and γδT cells sense tissue damage and trigger the mobilization of other immune cells. Neutrophils are followed by monocytes/macrophages and T cells. The relative amount of each cell type recruited is not represented. (B) Overview of the initial inflammatory phase following tissue injury. Chronological events are represented from left to right. Tissue damage is sensed by tissue-resident macrophages via DAMPs. Neutrophils are the first circulating immune cells recruited to the site of injury, promoting inflammation and monocyte/macrophage recruitment. The inflammation is initially maintained by pro-inflammatory M(IFN-γ) macrophages, before being eventually resolved with the help of M(IL-4) macrophages. (C) Overview of the immune mechanisms that can impair tissue healing or drive to scarring and fibrosis. M(IFN-γ) macrophages stimulate effector T cells in a positive-feedback loop. Effector T cells may also inhibit the regenerative capacity of tissue resident stem/progenitor cells via inflammatory cytokines. M(IL-4)-like macrophages with a pro-fibrotic activity encourage ECM protein deposition and subsequent fibrosis (scarring), preventing full regeneration of the original tissue. Pericytes increase immune cell mobilization and differentiate into scar forming myofibroblasts via growth factors such as TGF-β1. (D) Overview of the pro-regenerative immune mechanisms. A critical amount of macrophages displaying an anti-inflammatory/anti-fibrotic phenotype (e.g. M(IL-10)-like) contribute to regeneration through a crosstalk with Tregs, which in turn help sustain the anti-inflammatory/anti-fibrotic phenotype via secretion of anti-inflammatory cytokines such as IL-10. Tregs may also enhance the regenerative capacity of endogenous stem/progenitor cells through secretion of growth factors. Th2 cells induce/maintain anti-fibrotic/anti-inflammatory macrophages. Black arrows indicate a differentiation path or secretion of immune modulators/morphogens. Black dashed arrows indicate a hypothetical differentiation path. Red arrows indicate induction. Blue arrows indicate inhibition.

2.1. Danger signals

Directly after tissue injury, a local inflammation is induced in response to damage-associated molecular patterns (DAMPs, or alarmins) and pathogen-associated molecular patterns. Endogenous danger signals are typically released from necrotic or stressed cells and damaged ECM [2,3]. Well-known DAMPs include heat shock proteins (HSP), monosodium urate, high-mobility group box protein 1 (HMGB1), extracellular ATP, and nucleic acids including mitochondrial DNA. Inflammatory cytokines such as interleukin (IL)-1α and IL-33 can also work as DAMPs and are released passively from necrotic cells. In addition, fragments from

ECM components such as hyaluronic acid, collagen, elastin, fibronectin and laminin all stimulate inflammation [6,7].

Toll-like receptors (TLRs) and other types of pattern recognition receptors recognize danger signals and trigger inflammation via the activation of the transcription factors NF-κB or interferon-regulatory factors. TLRs activate tissue-resident macrophages and promote the expression of chemoattractants for neutrophils, monocytes and macrophages (Fig. 2A, B). They also induce the expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-1β and IL-6 [8,9]. Interestingly, inflammation in response to necrotic cells is mostly mediated by IL-1 receptor (IL-1R), which leads to NF-κB activation [10]. IL-33 also acts as a primary danger signal via the ST2 receptor [11]. However, the dominant danger signal varies in the context of the injury, including the location, magnitude, manner of cell death, and time point after the injury [3].

TLRs and IL-1R1 have been shown to negatively influence the repair of several tissues [12–22]. For instance, the harmful effect of TLR4 signaling is apparent in many organs, as seen by the protection of TLR4-mutant or deficient mice after hepatic, renal, cardiac, and cerebral ischemia-reperfusion [12–16]. Similarly, IL-1R1 signaling critically regulates infarct healing [17] and disruption of IL-1 signaling can improve the quality of wound healing [18,20]. In addition, it has been shown that IL-1R1/MyD88 signaling negatively regulates bone regeneration in the mouse by impairing the regenerative capacities of mouse MSCs [23]. While TLRs and IL-1R1 seem to be detrimental for many tissues, studies have shown that skin wound healing is impaired in mice deficient for various TLRs [24–26]. For example, TLR4 signaling helps wound healing through stimulation of transforming growth factor-β (TGF-β) and CC chemokine ligands (CCL)-5 expression [24]. Another endogenous TLR4 agonist, the extra domain A type III repeat of fibronectin (FNIII EDA) [27], has been reported to be overexpressed at sites of injury [28,29], and is known to influence skin repair [30]. For instance, wound healing in FNIII EDA knockout mice is abnormal [31].

Overall, it is clear that danger signals significantly influence the healing process at early stages. They are indeed necessary to induce inflammation, mainly via NF-κB, and they are also involved in neutrophil, monocyte and macrophage mobilization. Yet, in the case of ischemia-reperfusion and bone regeneration TLR and IL-R1 signaling seem to be detrimental.

2.2. Neutrophils and mast cells

Neutrophils are usually the first inflammatory cell recruited at a site of injury, enhancing host defense and wound detection while removing contaminants [32] (Fig. 2A, B). The recruitment of neutrophils requires changes on endothelium surface mediated by histamine, cytokines, and chemokines such as C-X-C motif ligand (CXCL) 8 that are released by tissue resident cells upon pattern recognition receptor and TLR activation. This will triggers a recruitment cascade involving the capture of free flowing neutrophils, followed by their transmigration from the vasculature to the tissue, facilitated by an increase permeability of the blood vessels at the injured site [32]. Neutrophils produce antimicrobial substances and proteases that help kill and degrade potential pathogens [33]. In addition, they secrete cytokines and growth factors such as IL-17 and vascular endothelial growth factor (VEGF)-A, which recruit and activate more neutrophils and other inflammatory cells, promote angiogenesis, and stimulate proliferation of cells such as fibroblasts, epithelial cells and keratinocytes (Fig. 2B) [32–34].

Neutrophils are also able to deploy neutrophil extracellular traps (NETs) [35], made of chromatin, proteins and enzymes, able to catch pathogens and either directly kill them or facilitate their phagocytosis. Yet, the formation of NETs (or NETosis) needs to be tightly regulated, since NETosis might impair the healing process. For example, there are evidences of delayed reepithelization in the case of diabetes where NETosis is enhanced [36]. This is consistent with the observation that neutrophil depletion might accelerate wound closure in diabetic mice [37].

Importantly, neutrophils exhibit anti-inflammatory capacities. They facilitate the recruitment of monocytes and macrophages, which phagocytize dying neutrophils and other cellular debris. Thus, neutrophils promote their own removal and thereby contribute to the resolution of inflammation (Fig. 2B) [32]. For example, following myocardial infarction, neutrophils help controlling, macrophages, polarization, which is a critical step for proper tissue repair [38]. Therefore, tightly controlling neutrophil mobilization and functions could be an interesting strategy to promote tissue repair and regeneration. For instance, pro-resolving mediators derived from omega 3 fatty acid have the ability to modulate neutrophil mobilization as well as their ingestion by macrophages [39].

Similarly to neutrophils, mast cells participate in the innate immune response by secreting an array of effector molecules to recruit eosinophils and monocytes. A large number of mast cells seem to be detrimental for tissue regeneration. For example, they enhance acute inflammation and promote scarring in the central nervous system [40]. Moreover, they persist at high numbers in chronic wounds [41]. Nevertheless, controlling mast cells to promote regeneration rather than repair and scarring should be tempered, since mast cells also produce anti-inflammatory mediators, suggesting alternative and dynamic functions for these cells during repair [40].

2.3. Monocytes and macrophages

In addition to their role as scavenger cells that phagocytise cellular debris, invading organisms, neutrophils and other apoptotic cells, macrophages actively regulate the tissue healing process [42]. A population of tissue macrophages resides in most tissues, but a large number of macrophages are recruited after tissue injury, and these often greatly exceed the population of tissue-resident macrophages [43]. The recruited and resident populations proliferate and undergo marked phenotypic and functional changes, in response to the tissue microenvironment. Importantly, macrophages are a source of various proteases, cytokines, growth factors, ECM components and soluble mediators promoting tissue repair, fibrosis, or regeneration [42,44,45].

Macrophages are differentiated from circulating monocytes which usually arrive at the damaged site 1–3 days after neutrophils (Fig. 2A) [46]. Their accumulation will often peak at 4–7 days after the injury, although elevated accumulations can be observed up to 21 days [47]. The two main blood monocyte subsets in the mouse are the $Ly6C^{hi}CX3CR1^{mid}CCR2^{+}$ ($CD62L^{+}CD43^{low}$) and the $Ly6C^{low}CX3CR1^{hi}CCR2^{-}$ ($CD62L^{-}CD43^{hi}$) monocytes [48] (human equivalents are the $CD14^{+}$ and the $CD14^{low}CD16^{+}$ monocytes). There is some evidence to suggest that the primary function of $Ly6C^{low}$ cells is to survey endothelial integrity [49,50]. By contrast, $Ly6C^{hi}$ monocytes represent "classical monocytes" that are recruited to sites of inflammation [48].

The two main chemokines/related receptors involved in the inflammation-dependent recruitment of monocyte subsets from blood, bone marrow and spleen are CCL2/CCR2 and CX3CL1/CX3CR1 (Fig. 2B) [51,52]. For instance, fibroblast, epithelial, and endothelial cells surrounding the injured tissue produce CCL2, in response to DAMPs and inflammatory cytokines. Interestingly, depending on the tissue, one or both monocyte subsets are recruited. For example, only $Ly6C^{hi}$ monocytes are recruited from the circulation in muscle injury models [53,54]. They first acquire

an inflammatory function and further mature into Ly6C^{low} macrophages with repair functions. However, after myocardial infarction, both monocyte subsets appear to home in the injured tissue at different stages of inflammation via CCR2 and CX3CR1, respectively [55]. The Ly6C^{hi} subset first infiltrates the infarcted heart and exhibits inflammatory functions, while the Ly6C^{low} subset is recruited at a later stage and stimulates repair by expressing high amounts of VEGF-A and by promoting deposition of collagen.

Driving the recruitment of different monocyte populations, both CCR2 and CX3CR1 appear to be essential for proper healing in several tissues. For example, *Cx3cr1*$^{-/-}$mice display reduced levels of α-smooth muscle actin and collagen, reduced neovascularization as well as delayed healing in skin wounds [56]. Similarly, the loss of CX3CR1 leads to delayed skeletal muscle repair [57]. Moreover, deficiency in the CCL2–CCR2 axis appears to impair muscle and skin repair [58]. For instance, Eming and colleagues have shown that CCR2 is critical for the recruitment of Ly6C^{hi}CCR2^{+} monocytes to skin wounds, leading to proangiogenic macrophages crucial for vascularization [59]. Interestingly, the study showed that macrophages are the main source of VEGF-A in early tissue repair.

Pro-inflammatory macrophages – the so-called "M1" macrophages – may become polarized towards a variety of alternatively activated anti-inflammatory "M2" macrophages [42]. Although pro-inflammatory and anti-inflammatory macrophages are the two most frequently investigated phenotypes in studies of tissue healing, macrophages exhibiting tissue repair, pro-fibrotic, anti-fibrotic, pro-resolving, and tissue regeneration characteristics are also commonly mentioned in the literature [42]. Indeed, the M1 and M2 nomenclature originate from *in vitro* characterization where the M1 phenotype is produced by exposure to IFN-γ and TNF-α, while the M2 phenotype is produced by IL-4 or IL-13 [60]. In this review, we adopted the new classification system proposed by Murray et al. [61] where nomenclature is linked to the activation standards i.e., M(IFN-γ), M(IL-4), M(IL-10), and so forth.

Generally, M(IL-4) macrophages are considered as tissue repair macrophages, since they express several wound healing factors such as arginase, ECM components and growth factors such as VEGF-A, platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF) [42,56,60] (Fig. 2B). Yet, the mechanisms that drive macrophages to adopt various tissue repair phenotypes *in vivo* are still under intense debate [42,60]. Indeed, macrophage phenotype associated markers may be expressed simultaneously, making *in vivo* characterization even more challenging [63]. In addition to cytokines, microRNAs (miRNA), which control messenger RNA translation and degradation (e.g. messenger RNAs of cytokines and transcription factors), are most likely critical regulators of macrophage polarization [62,63]. More specifically, miR-9, miR-127, miR-155, and miR-125b have been shown to promote M(IFN-γ) polarization while miR-21, miR-124, miR-223, miR-34a, let-7c, miR-132, miR-146a, and miR-125a-5p support M(IL-4) polarization in macrophages by targeting various transcription factors and adaptor proteins [62,63].

While inflammatory macrophages can exacerbate tissue injury and impair tissue healing, persistent activation or sustained mobilization of M(IL-4) macrophages has been hypothesized to contribute to the development of pathological fibrosis [42] (Fig. 2C). For example, the pro-fibrotic function of M(IL-4) macrophages has been attributed to their production and activation of TGF-β1 in models of pulmonary fibrosis [64]. In addition to producing pro-fibrotic mediators, M(IL-4) macrophages have been shown to directly enhance the survival and activation of myofibroblasts, which are key cells producing ECM in all organs [65]. Pro-fibrotic M(IL-4) macrophages also produce significant amount of matrix metalloproteinases (MMPs), and some of which serve as essential drivers of fibrosis [66].

Macrophages may also be anti-inflammatory/anti-fibrotic and they are thought to be critical for the resolution of most tissue injury inflammation responses. IL-10 – an immunoregulatory cytokine produced by a variety of cell types, including T helper 2 cells (Th2), regulatory T (Treg) cells and macrophages – is known to function as a critical anti-inflammatory mediator [67]. In addition, anti-inflammatory macrophages regulate the development and maintenance of IL-10- and TGF-β1-producing Tregs, which contribute to the resolution of inflammatory responses in multiple tissues (Fig. 2D) [68]. Nevertheless, beside the expansion of IL-10-induced anti-inflammatory macrophages, other mechanisms have also been shown to trigger anti-inflammatory macrophages [42]. For example, IL-6 and IL-21 have been found to enhance IL-4R expression on macrophages and contribute to the development of anti-inflammatory and anti-fibrotic macrophage function following stimulation with IL-4 or IL-13 [69,70].

Interestingly, it has been recently demonstrated that macrophages are critical for the regeneration (i.e. the full restoration of the tissue function) of various tissues [71–73]. For example, Godwin and colleagues found that macrophages are essential for limb regeneration in adult salamanders [71]. Moreover, mice can regenerate cardiac tissue until seven days post-birth and it has been demonstrated that monocytes and macrophages are required for the cardiac regeneration process. Remarkably, profiling of cardiac macrophages from regenerating and non-regenerating hearts indicated that neonatal macrophages have a unique polarization that does not fit into M(IFN-γ) or M(IL-4) phenotypes [73].

Importantly, it remains unclear whether an individual macrophage (recruited or tissue-resident) is capable of adopting all the phenotypes at different time in response to the injured tissue microenvironment, or if distinct subsets of monocytes and macrophages are committed to adopt the various phenotypes [42,60]. For instance, in several tissues such as the central nervous system and the liver, macrophages switch from a pro-inflammatory phenotype to a repair phenotype where IL-4, IL-10 and phagocytosis play critical roles in the conversion [74–76]. In the context of skin injury, chemokines (e.g. CX3CL1) drives circulating CX3CR1^{hi} monocytes traffic into the damaged site. The CX3CR1^{hi} monocytes become M(IL-4)-like macrophages and secrete factors such as VEGF-A, TGF-β [56], IL-13, IL-10 and several chemokines [77].

Overall, monocytes and macrophages can exacerbate inflammation, promote tissue repair and fibrosis, or drive regeneration. While the detailed mechanisms regulating macrophage functions during tissue healing are still unclear, their critical role in the repair and regeneration processes marks them as a primary target when designing regenerative strategies.

2.4. Pericytes

Pericytes are ubiquitous mural cells of blood microvessels, which facilitate the initial extravasation of immune cells from the blood [78]. Pericytes are also a source of stem/progenitor cells and they secrete multiple growth factors and cytokines, as well as other soluble mediators [79] (Fig. 2D). For example, they contribute to skeletal muscle regeneration by driving immune cells to cross the endothelium [80] and they are most likely a source of myogenic precursors [80]. Pericytes also contribute to tissue healing by promoting angiogenesis at the damaged site [79]. For instance, injection of pericytes into mouse cardiac tissue after infarction improves the healing process, by reducing scar formation, fibrosis and cardiomyocyte apoptosis via secretion of angiogenic factors and miRNA [81]. These examples demonstrate that pericytes are pro-regenerative cells, but they are also source of scar-forming myofibroblasts in several organs, including skin, liver, and in the central nervous system [1,79]. In addition, pericytes interact with the immune cells involved in the scarring process.

For example, they induce the mobilization of Ly6C^{hi} monocytes that further stimulate scar formation by secreting factors such as TGF-β1, TNF-α, and PDGFs (Fig. 2C). These factors induce pericytes to change their morphology leading to vascular permeability, proliferation and expression of tissue inhibitors of metalloproteinases (TIMPs) [82]. Therefore, pericytes have the capacity to support regeneration, but in acute or chronic inflammation their regenerative function can switch to a fibrotic function. Consequently, one should design strategies to promote the regenerative capacity of pericytes (i.e. differentiation into functional tissue cells), while avoiding promotion of their differentiation into myofibroblasts.

2.5. Dendritic cells

In a manner similar to macrophages, dendritic cells (DCs) will phagocytise particles and process danger signals at the injury site. Although their precise role during tissue repair and regeneration remains not fully understood [83], studies show that they play an important role in the tissue healing process [26,83–85]. For example, it has been shown that plasmacytoid DCs sense skin injury via host-derived nucleic acids (recognized by TLR7 and TLR9) and promote wound healing through type I interferons [26]. Burn wound closure is also significantly delayed in DC-deficient mice [83]. The impaired wound healing seems to be associated with significant suppression of early cellular proliferation, granulation tissue formation, wound levels of TGF-β1 and formation of blood vessels. In addition, in a myocardial infarction model, DC-depleted mice show impaired ventricular functions and remodeling, with particularly high levels of inflammatory cytokines along with an unbalanced M(IFN-γ):M(IL-4) macrophages ratio strongly tilted towards M(IFN-γ) [85]. DCs most likely act as an immunoregulator during tissue healing through control of macrophages homeostasis.

2.6. T cells

Growing evidence points towards T cells playing a crucial role in tissue repair and regeneration. While interesting mechanisms have been revealed, the exact function of the different T cell types and subsets and their level of accumulation at injury sites are largely unknown and seem to vary from tissue to tissue. The majority αβT cell fraction appears to have both pro- and anti-regenerative sub-populations. Meanwhile, the minority tissue resident γδT cell fraction has been widely reported as being pro-regenerative [86–89].

T cells are capable of secreting a diverse range of cytokines and growth factors, which have beneficial or inhibitory effects on tissue healing (Fig. 2C, D). In the context of bone, there is evidence that both CD4$^+$ (T helper 1, Th1) and CD8$^+$ (cytotoxic) T cell subsets inhibit regeneration [90,91]. For example, fracture healing is accelerated in *Rag1*$^{-/-}$mice (a mouse model without functional T and B cells) [92] or when CD8$^+$ T cells are actively depleted [90]. On a mechanistic level, it has been demonstrated that T cells inhibits MSC-driven bone formation in the mouse via IFN-γ and TNF-α [91]. Similar research in humans showed that secretion of IFN-γ and TNF-α by effector memory CD8$^+$ T cells can result in delayed osteogenesis and fracture healing [90]. On the other hand, studies have shown that CD4$^+$ Tregs are critical for the repair and regeneration of several tissues including skin [93], bone [91,94], lungs [95–97], kidney [98,99], skeletal muscle [100,101], and cardiac muscle [102]. For example, after damage to mouse skeletal muscles, Tregs can comprise up to 50% of the T cell population between day 14 and 30 [100]. The presence of Tregs results in the production of arginase [103] and anti-inflammatory cytokines such as IL-10 and TGF-β [94]. These secreted factors provide an anti-inflammatory microenvironment conducive to repair and polarization of macrophages [94]. Even as conventional T cells move away, Treg levels remain elevated. This may be because Tregs that reside in visceral adipose, muscle and lamina propria express epithelial growth factor receptor (EGF-R) [104,105]. The expression of EGF-R allows the growth factor amphiregulin secreted by mast cells to maintain Tregs at the damaged site [104]. Once present, Tregs proliferate and upregulate amphiregulin secretion, which is necessary for regeneration [100].

The γδT cells are also important in the tissue healing process. For example, both humans and mice do not heal skin wounds as fast or effectively in the absence of γδT cells [106]. Functionally, the pro-repair insulin-like growth factor-1 (IGF-1) is produced by both mouse [107] and human [108] γδT cells. In the context of tissue healing, the dendritic epithelial γδT cells (DETCs) are the most well characterized γδ subset [88]. DETCs have an unusual dendritic-like morphology in the mouse skin, and they respond within hours to skin tissue damage by secreting chemokines and TNF-α to attract macrophages [88]. Additionally, DETCs accelerate tissue repair by secreting growth factors and cytokines such as IGF-1, KGF-1 (FGF-7), KGF-2 (FGF-10), IL-22, and IL-17A [88]. For instance, it has been shown that γδT cells peak between 2 and 7 days after bone injury in the mouse and secrete IL-17A, which enhance osteoblast functions [87]. Additionally, γδ-derived IL-22 prompts proliferation and migration of epithelial cells in various tissues [109]. Overall, γδT cells play both a central role in recruiting innate immune cells as well as directly stimulating tissue growth.

We have made significant headway in understanding the importance of T cells during tissue repair and regeneration, in particular Tregs and γδT cells. Treg and γδT cells secreted growth factors and cytokines are most likely critical for to orchestrate tissue healing, particularly in skin and muscle. Nevertheless, the mechanisms by which the different T cell types and their respective subsets modulate the immune response to tissue injury are still very elusive. In addition, T cells probably directly interact with tissue resident stem or progenitor cell populations, and this could be a useful niche to exploit for designing new regenerative strategies.

2.7. B-cells

There is little available evidence on the role of B cells in tissue healing. Given the origin of B cells within the bone marrow, it would be expected that there would be cross talk between B cells and bone tissue [110]. For example, IgM$^+$ B cells are important in repair by secreting osteoprotegerin to accelerate bone regeneration [111]. Interestingly, while CD4$^+$ T cells help upregulate osteoprotegerin via the CD40/CD40L pathway, CD8$^+$ T cells in contrast inhibit osteoprotegerin expression [111]. As noted above, mice deficient in both T and B cells have faster bone healing, suggesting depletion of the adaptive immune system as a promising strategy to augment bone regeneration. However, we would argue that there is still much to be discovered regarding the role of B cells in the repair and regeneration of various tissues.

3. Promoting tissue regeneration by modulating the immune system

In the first part of this review, we have seen that the immune system greatly influences tissue repair and regeneration in both negative and positive fashion. Therefore, controlling the immune regulations of tissue healing is becoming an attractive avenue in regenerative medicine, and the design of regenerative strategies may progress in parallel with our understanding of the crosstalk between immune components, stem/progenitor cells and the tissue healing process. In the next sections, we highlight different

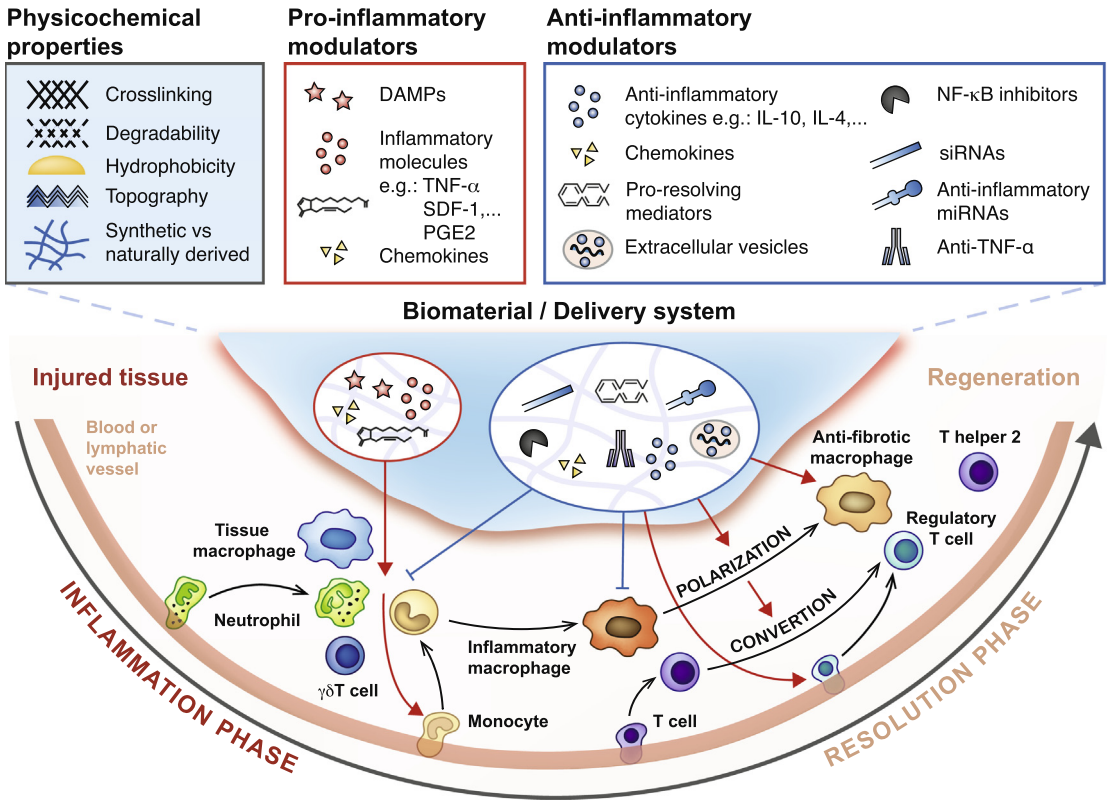

Fig. 3. Strategies based on biomaterials and drug delivery systems to promote tissue regeneration by controlling the immune system. Biomaterial-based strategies aiming at improving the healing process through immunomodulation can be achieved either by the biomaterial itself and/or by the delivery of immunomodulators. Strategies most commonly aim either at the delivery of pro-inflammatory modulators to initiate the healing process or the delivery of anti-inflammatory modulators to promote the resolution phase via anti-inflammatory/anti-fibrotic macrophages. More complex strategies rely on a sequential delivery of pro-inflammatory and anti-inflammatory molecules to exert a more comprehensive control over the tissue healing process. Black arrows indicate a differentiation path. Red arrows indicate induction. Blue arrows indicate inhibition. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

approaches that attempted controlling the immune system to promote tissue repair or regeneration. In many cases, these approaches are based on biomaterials or use biomaterials as delivery systems for immune modulators (Fig. 3).

3.1. Immune modulation by the physicochemical properties of biomaterials

Implanted biomaterials can have a significant intrinsic effect on the immune system and macrophage polarization, either promoting or reducing inflammation depending on their physicochemical properties. The form that the biomaterial takes (solid, hydrogel or micro/nanoparticles), the level of crosslinking and the degradability, the hydrophobicity, the topography, and the nature of the biomaterial (synthetic vs naturally derived) are important parameters to consider (Fig. 3) [112,113]. Synthetic biomaterials that have been used to modulate the immune response following tissue injury are for example poly lactic-co-glycolic acid (PLGA), poly(lactic acid) (PLA), and polyethylene glycol (PEG). Naturally derived biomaterials are for instance decellularized tissues such as human or porcine skin or porcine small intestine submucosa (SIS), or fabricated scaffolds made of natural molecules such as collagen, fibrin, hyaluronic acid, chitosan, alginate or silk.

Illustrating the importance of the biomaterial crosslinking, scaffolds with a high level of crosslinking usually drive a predominantly inflammatory macrophage response [112,113]. For example, it has been demonstrated that SIS implantation in rat preferentially induced anti-inflammatory macrophages while a carbodiimide crosslinked form of SIS induces predominantly inflammatory macrophages [114]. Similarly, macrophages seeded on non-crosslinked porcine dermis or non-crosslinked porcine SIS, produces lower levels of IL-1, IL-6, and IL-8 compared to macrophages seeded on chemically cross-linked porcine dermis [115].

The surface chemistry also appears to influence macrophage adhesion and their cytokine secretion profile. For example, neutrally charged hydrophilic-modified polymers have been shown to promote less macrophage and less foreign body giant cell formation compared to hydrophobic and ionic surfaces [116]. Although there were fewer cells on the hydrophilic/neutral surface, the macrophages were further activated to produce significantly greater amounts of cytokine (IL-1, IL-6, IL-8, and IL-10) than hydrophobic and ionic surfaces [116].

When designing a biomaterial, modulating the surface topography is an interesting method to regulate the cellular response via control of cell shape and elasticity. The modulation of macrophage function, phenotype and polarization to varying topography has been a subject of research for several decades [112]. Studies on the role of topography on macrophage polarization strongly suggest an advantage of stimulating macrophage elongation for promoting anti-inflammatory polarization [117]. This can be achieved by micro-patterning the surface and to control attachment, or could be achieved by patterning macrophage ligands on the surface to promote elongation of cells [112].

A number of naturally derived biomaterials such as high molecular weight hyaluronic acid [118] and chitosan [119], which have radical oxygen species-scavenging properties, have intrinsic anti-inflammatory properties. Nevertheless, in the case of most

biomaterials, loading or functionalization of the biomaterial with anti-inflammatory molecules is necessary to modulate the inflammatory microenvironment. Naturally derived biomaterials such as collagen and fibrin are ideal for releasing immune modulators through enzyme-mediated degradation. On the other hand, synthetic materials may allow for increased control over degradation and release kinetics of therapeutics, with the caveat that the biomaterial itself and its degradation products should cause a minimal response when implanted [115].

3.2. Immune modulation by decellularized ECM

Excised tissues can be processed to separate cells from the ECM, leaving only a decellularized ECM scaffold. The structure of these natural scaffolds influences numerous cellular processes and can be used to create a pro-regenerative environment [120]. Moreover, with the ECM proteins being highly conserved across species, xenografts are usually well tolerated [121], limiting the risk of undesired inflammation which could interfere with the regulation of the immune environment. Indeed, among other properties, decellularized ECM has shown to modulate the wound immune microenvironment through macrophage polarization [114], with the ability to direct macrophages towards either an M(IFN-γ) or M(IL-4) phenotype. This immune modulation usually depends on the composition and structure of the scaffold. Although the exact underlying mechanism is still not fully elucidated [122], a recent study suggests that the effects could be carried out by matrix-bound microvesicles (MBVs) embedded in the ECM [123]. The study by Badylak and colleagues showed that MBVs were biologically active and were partially responsible for the effect of the scaffold. Indeed, after isolation from urinary bladder matrix, MBVs were able to stimulate neurite extension on neuroblastoma cells. A potential mediator of this activity are miRNAs present within MBVs. Interestingly, although a certain number of miRNA were conserved across multiple MBVs of different source, a significant amount was tissue-specific and could partially explain the different effects induced by decellularized scaffolds depending on their tissue of origin.

Interestingly, the ability to control inflammation through macrophage polarization allows xenograft of acellular ECM to be more beneficial than an autologous transplantation in some cases. For example, in a model of tendon reconstruction in mice, the use of decellularized urinary bladder matrix induced a greater migration of progenitor cells towards reconstructed tendons compared to autologous grafts [124]. This improved mobilization of progenitor cells seems to be attributed to an anti-inflammatory M(IL-4)-like response induced by the decellularized ECM scaffold. Indeed, it has been extensively shown that transplantation of acellular scaffolds usually results in an M(IL-4)-like response with less scarring compared to cellular scaffolds [122]. In addition, it has been recently demonstrated that tissue-derived ECM scaffolds induce a pro-regenerative immune environment through a robust Th2 immune response, which drive macrophage polarization towards an M(IL-4) phenotype via IL-4 [125].

Importantly, the type of response induced by a decellularized ECM scaffold highly depends on the source tissue were the ECM was harvested. Indeed, a study comparing the macrophage response after being exposed to ECM derived from different types of tissue showed a very heterogenous behavior [126]. In this study, SIS, urinary bladder matrix, brain ECM, esophageal ECM, and colonic ECM all induced an M(IL-4) response while dermal ECM induced an M(IFN-γ) phenotype. Interestingly, ECMs derived from liver, and skeletal muscle did not induce a particular macrophage phenotype.

Decellularized ECMs also present an interesting option for the delivery of immunomodulatory molecules. For instance, decellularized bones have been used for the sequential release of two types of cytokines, the pro-inflammatory IFN-γ and the anti-inflammatory IL-4 [127]. This sequential release promoted macrophage transition from a M(IFN-γ) to M(IL-4) phenotype and enhanced vascularization of the bone scaffolds in a murine subcutaneous implantation model.

3.3. Delivery of inflammatory molecules

There is a large emphasis on enhancing tissue repair by down-regulating unwanted inflammation. However, pro-inflammatory molecules including danger signals and pro-inflammatory cytokines are necessary to start the tissue healing program. For instance, the delivery of heat shock protein 70, an endogenous agonist of TLR2 and TLR4 [128], accelerates wound healing by up-regulating macrophage-mediated phagocytosis [129]. Similarly, activation of TLR9 using CpG has been shown to promote skin repair in primates [130]. These examples demonstrate that the principle of using pro-inflammatory molecules to treat tissue damage could work in some cases (Fig. 3). Indeed, the inflammatory chemokine stromal cell-derived factor-1 (SDF-1, CXCL12) and prostaglandin E2 (PGE2) have been extensively explored in tissue repair and regeneration.

3.3.1. SDF-1

SDF-1 is an inflammatory and pro-angiogenic chemokine that has been shown to be very important in the tissue healing process [131], in particular by its capacity to mobilize progenitor cells [132]. For instance, both human and mouse MSCs express CXCR4, a SDF-1 receptor, allowing the cells to traffic towards SDF-1 [133,134]. A large number of studies have used biomaterials such as silk-collagen [135], gelatin [136], alginate [137], PEGylated fibrin [138], poly(lactic-co-glycolic acid) [139], and thiol functionalized sP(EO-stat-PO) [140] to deliver SDF-1 in a controlled manner, both to increase angiogenesis and recruit $CXCR4^+$ cells, including macrophages [141], hematopoietic stem cells [132] and MSCs [139]. Biomaterials delivering SDF-1 have been used for many tissue types and the usefulness of this strategy has been demonstrated in tendons [135], cardiac muscle [138,140], skin [136] and liver models [132]. Nevertheless, one challenge to using SDF-1 is its sensitivity to protease, as the cytokine is cleaved by MMP-2 and serine exopeptidase CD26. This unwanted protein degradation can be overcome by modifying the MMP-2/CD26 cleavage sites or by codelivering enzymes inhibitors such as saxagliptin [132]. A second concern may be that SDF-1 is implicated in macrophage-driven hypertrophic scar formation. Indeed, cells such as mouse lung fibrocytes and pro-fibrotic pericytes have also been shown to traffic towards SDF-1 *in vivo* [79,97]. Therefore, appropriate SDF-1 dosing is important when designing therapies, to avoid induction of fibrosis.

3.3.2. PGE2

Prostaglandin E2 (PGE2) is part of a family of pro-inflammatory lipid molecules known as prostanoids [142]. PGE2 and its multiple receptors (EP1, EP2, EP3 and EP4) have been involved in both pro- and anti-regenerative functions. For example, elevated levels of PGE2 are found in periodontal disease [143]. Conversely, PGE2 can increase bone formation [142,144] and angiogenesis [145]. Within the immune system, PGE2 can induce proliferation of T cells and cause their apoptosis [142]. Interestingly, while being pro-inflammatory, PGE2 has also been shown to inhibit proliferation and skews the immune response to Th2 [142] by inhibiting IL-12 [146], IFN-γ [147] and IL-2 [148] secretion by human lymphocytes.

While PGE2 can be beneficial for tissue healing, PGE2 therapy requires multiple doses and has significant side effects, making it

a poor therapeutic [144,149]. As such limiting PGE2 locally using biomaterial delivery systems would be better than repeated systemic administration of PGE2. For example, PGE2 in a cholesterol bearing pullulan nanogel was effective in building bone in mice [150]. Nanogels could be further improved by replacing PGE2 with an agonist that only binds one of its four receptors. Two studies using an BMP-2/EP4 agonist combination in either a PEG nanogel [149] or polylactic acid gel [151] were successful in inducing bone repair or mineralization respectively in mice. Thus, using an agonist to a specific PGE2 receptor such as EP4 in combination with growth factors slowly released via a biomaterial may be an effective therapy.

3.4. Delivery of anti-inflammatory molecules

Although inflammation at the site of tissue injury is necessary to kick-start the healing response, its resolution is crucial to advance the healing process and to restore tissue integrity. The pro-inflammatory function of macrophages is essential during the early stages of inflammation, but proper tissue healing requires macrophages to be polarized towards an anti-inflammatory phenotype. The pro-resolving activity of macrophages notably includes the development and maintenance of Tregs. Tregs in turn contribute to creating an anti-inflammatory environment beneficial to tissue repair and help sustain the anti-inflammatory phenotypes of macrophages (Fig. 2D). The mechanisms inducing the passage from a pro-inflammatory state to a resolution state naturally exist, but therapeutic strategies aiming at promoting this transition can further improve the healing process (Fig. 3). For example, polymer particles fabricated from poly (cyclohexane-1,4-diylacetone dimethylene ketal) were loaded with an inhibitor of p38, a mitogen-activated protein kinases important for immune cell activation, to diminish the post-infarction inflammatory response in the myocardium [152]. In a myocardial infarction model, the particles significantly reduced superoxide and TNF-α production, and resulted in a reduction of fibrosis as well as improved cardiac function.

3.4.1. Pro-resolving mediators

Resolvins, protectins, lipoxins and maresins secreted by phagocytes, are specialized pro-resolving mediators derived from omega 3 fatty acids [153,154], limiting both the recruitment of neutrophils and their ingestion by macrophages [39]. For example, pro-resolving mediators upregulate the expression of CCR5 (a receptor for inflammatory chemokines such as CCL3 and CCL5) by senescent neutrophils and activated T cells. Thus, $CCR5^{+}$ apoptotic leukocytes sequester inflammatory chemokines and act as terminators of their signaling during the resolution of inflammation [155]. A resolvin-based strategy has already proved to be efficient at promoting wound healing in a model of obese diabetic mice through enhanced resolution of peritonitis [156]. Similarly, administration of protectin on wounds in the same diabetic mouse model also improved reepithelization and the formation of granulation tissue as well as innervation [157]. Injections of resolvin and lipoxin have also been shown to be able to control the macrophage polarization induced after a chitosan scaffold implantation [158]. Indeed, although chitosan usually induces inflammatory macrophages when the degree of acetylation exceeds 15% [159], injections of lipoxin or resolvin were able to shift the polarization balance towards a anti-inflammatory phenotype in a mouse air-pouch model.

3.4.2. Inhibitors of TNF-α

The pro-inflammatory activity of M(IFN-γ) macrophages is largely mediated by the release of TNF-α. While this cytokine has been shown to positively regulate tissue repair and regeneration in some situations, its excess can impair the healing process. For example, pathological levels of TNF-α may induce osteoclastogenesis (via T cell secretion of RANKL which activates RANK on osteoclasts) resulting in more bone reabsorption than osteogenesis. Thus, strategies aiming at blocking the activity of TNF-α have been proposed to diminish the effect of the pro-inflammatory macrophages. Local delivery of common painkillers including aspirin [160], ibuprofen [161] and pentoxifylline [162] have shown encouraging results in reducing TNF-α. For example, simply delivering aspirin locally with hydroxyapatite/tricalcium phosphate ceramic particles could reduce TNF-α and prevent apoptosis of transplanted MSCs, resulting in more bone regeneration [91]. Other strategies include directly targeting TNF-α with TNF-α antibodies. For example, a delivery system based on chitosan/collagen scaffold has been developed [163], in which a glucose-sensitive delivery system was capable of releasing TNF-α antibodies upon increase of glucose level in a diabetic rat model, a condition often associated with alveolar bone destruction and high level of TNF-α. The system successfully reduced inflammation and promoted alveolar bone healing. Other studies have used hyaluronic acid as a delivery vehicle for anti-TNF-α. Hyaluronic acid can bind CD44 on macrophages and thus provide the anti-TNF signal directly to the cell producing the cytokine. For example, hyaluronic acid plus a monoclonal antibody for TNF-α was effective at inducing early healing in the rats after a burn [164].

Although many studies have focused on inhibition of TNF-α as a therapy to overcome unwanted inflammation and accelerate tissue healing, it should be noted that TNF-α might be a useful cytokine to help begin the healing process in some tissues. For example, in a rat model, pre-stimulation of MSCs with TNF-α increased their engraftment to myocardial infarct [165]. Additionally, TNF-α enables mobilization of human and mouse MSCs into damaged tissues [166,167]. After bone fracture in the mouse, TNF-α levels peak at 24 h post-injury, and help recruit pro-regenerative cells such as MSCs [167]. A second wave of TNF-α expression peaks at about four weeks after injury and is necessary for endochondrial bone formation [167]. In other tissues, TNF-α could also play a positive role as it helps stimulate production of BMP-2 in the context of cardiac [165] and skin [168] repair.

3.4.3. Inhibitors of the NF-κB pathway

Many DAMPs and inflammatory cytokines such as IL-1 and TNF-α induce the NF-κB pathway and there is a growing body of evidence that inhibiting NF-kB may be a viable option to accelerate the healing of some tissues. For example, mice deficient in IL-1 receptor antagonist (IL-1Ra) show delayed wound healing due to higher neutrophil recruitment and subsequent NF-κB activation in fibroblasts resulting in negative regulation of the pro-repair TGF-β pathway [169]. In addition, targeting NF-κB may aid bone regeneration. For instance, inhibiting NF-κB in mouse osteoblasts can increase bone density in an induced osteoporosis model [170]. Moreover, it was shown that inflammatory cytokines such as TNF-α and IL-17 reduce osteogenesis of mouse MSCs. These cytokines impair the Wnt/β-Catenin signaling in MSCs, which is critical for osteogenesis [171]. Co-delivering MSCs on apatite-coated PLGA scaffold with a small inhibitor of IKKβ (which is a subunit in the kinase enzyme complex part of the upstream NF-κB signaling) resulted in much more bone formation *in vivo* compared to MSCs delivered without inhibitor. Similarly, it was shown that IL-1β signaling through the IL1-R1/MyD88 pathway inhibits mouse MSC proliferation, migration and differentiation towards osteoblasts [23]. Indeed, MSC response to growth factor and Wnt signaling were impaired by IL-1β, due to AKT dephosphorylation and β-catenin degradation. Mouse calvarial defect treated with IL1Ra or a MyD88 inhibitor designed to be covalently incorporated into fibrin hydrogels and to translocate into cells following

hydrogel remodeling by proteases significantly improved bone regeneration driven by MSCs [23]. Taken together, these studies indicate that NF-kB inhibition could have a dual positive effect of reducing inflammation while increasing regeneration driven by MSCs.

3.4.4. Anti-inflammatory cytokines

Anti-inflammatory cytokines such as IL-4 and IL-10 are critical for proper tissue repair and regeneration, since they are involved in M(IFN-γ) to M(IL-4) macrophage switching [42]. In particular, mouse models have convincingly shown that IL-10 is necessary for scar-free healing [172]. Indeed, fetal mice are able to repair cutaneous damage scar free via IL-10 dependent mechanisms, and this ability can be replicated in adult mice that overexpress IL-10 [172]. The same effect seems to happen in the heart. It has been shown that fibrosis after infarct in the mouse is considerably reduced, when IL-10 is delivered through heparin-based coacervate [173]. For regenerative medicine purposes, IL-10 has been mostly delivered using plasmid DNA and virus vectors [176], but IL-4 is often delivered as a protein to induce M(IL-4) macrophage polarization. For example, slow release of IL-4 conjugated to a bone scaffold via biotin-streptavidin can enhance M(IL-4) macrophage polarization [127]. In a rat model of *in vivo* peripheral nerve damage, IL-4 delivered via injectable agarose hydrogel was effective in increasing the number of M(IL-4) macrophages [174]. Interestingly, IL-4 delivery via this method resulted in much more axons being regrown after three weeks compared to the controls, suggesting controlled release of IL-4 may help with peripheral nerve repair via M(IL-4) macrophages. In a different context, it was shown that IL-4 delivery could potentially reduce bone degradation after joint replacement [175]. The study showed that polyethylene particles could degrade mouse calvarias *in vitro*, but this process was ameliorated via addition of IL-4 [175]. This study suggests that incorporating IL-4 or similar anti-inflammatory cytokine to implanted materials may prevent unwanted side effects of implants. Overall, the delivery of IL-4 may enhance tissue regeneration in various situations via M(IL-4) macrophage induction. What is remaining to be explored is the direct effect that IL-4 could have on T cells.

Another interesting anti-inflammatory cytokine is TGF-β1, which is necessary for tissue repair at the earliest stages [176], although the molecule has inflammatory or anti-inflammatory effects depending on the cell type it signals. For example, TGF-β1 can suppress lymphocyte proliferation as well as activity and can help to induce immune-suppressive Tregs [177]. Nevertheless, the cytokine is also highly involved in scar formation [176]. However, TGF-β has three isoforms (TGF-β1, 2 and 3) and there is evidence showing that TGF-β3 can be harnessed to accelerate regeneration and avoid scarring [176]. Indeed, TGF-β3 simply injected alone on incisional wounds in human patients was able to slightly, but noticeably reduce post-operative scarring [178]. The design of an optimal delivery system for TGF-β3 may therefore improve its anti-fibrotic capacity in humans.

3.4.5. siRNA

Small interfering RNA (siRNA)-mediated gene silencing offers an alternative therapeutic strategy to antibodies and chemical-based inhibitors. A number of studies have demonstrated the potential of RNA interference to suppress pro-inflammatory pathways and inflammatory cytokines [176,179–182]. The major challenges for therapeutic use of siRNAs are to develop methods for delivering siRNAs to the desired cell types *in vivo* and to escape from the endosomal compartment [182].

PLGA particles have been used to deliver TNF-α siRNA for treating inflammation associated with rheumatoid arthritis. In a mouse model of rheumatoid arthritis, the particles resulted in a reduction of TNF-α production and inflammation in the joint. Similarly, PLGA particles have been used to deliver polyetherimide(PEI)-conjugated FcγRIII-targeting siRNA to reduce inflammation [179]. The system proved to be efficient in a rat model of temporomandibular joint inflammation with reduction of IL-1 and IL-6. In a remarkable study, a lipid nanoparticle was used to deliver a therapeutic siRNA that reduced the accumulation of CCR2 pro-inflammatory monocytes to inflamed tissue [180]. The siRNA targeting CCR2 was administered systemically, and shown to reduce the infarct size in a myocardial infarction model, reduce inflammatory cells in atherosclerotic lesion, improve the survival of pancreatic islet allografts, and reduce tumor volume. Similarly, nanoparticle-based RNA interference that effectively silences five key adhesion molecules for arterial leukocyte recruitment has been used to prevent complications after acute myocardial infarction [181]. Simultaneously encapsulating siRNA targeting intercellular cell adhesion molecules 1 and 2, vascular cell adhesion molecule 1, and E- and P-selectins into polymeric endothelial-avid nanoparticles reduced the recruitment of neutrophil and monocyte after myocardial infarction into atherosclerotic lesions and decreased matrix degrading plaque protease activity. The five-gene combination RNA interference also curtailed leukocyte recruitment to ischemic myocardium. Overall, these studies emphasize the potential of siRNA as a therapeutic to control the immune system and to reduce the detrimental effect of excessive inflammation during the tissue healing process.

3.4.6. miRNA

miRNAs play an important role in immunity [183–185] and tissue healing [186–188]. Their ability to regulate the immune system on multiple levels is of particular interest here as they appear to be involved in the development and functions of hematopoietic stem cells, as well as innate and adaptive immune cells. For example, miRNAs can direct macrophages polarization through targeting of the IRF/STAT pathway, promoting inflammation and its resolution [62]. Moreover, miRNAs can induce Tregs [189] and regulate many other aspects of the T cell response [190] via modulation of TCR signaling [191] and T helper cells plasticity [192]. For instance, miR-181a has the ability to initially help activate mature T cells through increased TCR signaling sensitivity, but also to later repress this activation through downregulation of CD69, a promoter of T cell proliferation [196].

Although therapeutic strategies based on the delivery of miRNAs are still scarce, studies have shown that their use can be beneficial to tissue healing [193]. For example, in a rat skeletal muscle injury model, the combined injection of three different miRNAs improved muscle regeneration while preventing fibrosis [194]. Nevertheless, direct injections of miRNA present limitations such as *in vivo* stability or biodistribution, which could be overcome by the development of advanced delivery systems [193,195]. As for siRNA, biomaterial-based delivery systems are necessary to optimize the delivery of miRNA [196]. For instance, hydrogels have been successfully used for *ex vivo* delivery of miRNAs to cells in 3D culture [197] and could provide an alternative to soluble injections for *in vivo* delivery at a specific site. Nanoparticle are also a good option for *in vivo* delivery of miRNA. For example, delivery of miR-146a using PEI nanoparticles was able to inhibit renal fibrosis through suppression of the infiltration of $F4/80^{+}$ macrophages [198]. Currently, options are also being pursued to modulate miRNAs signaling *in vivo*, either by overexpression or inhibition. However, improvements in delivery methods of the modulators are also required [193].

3.5. Extracellular vesicles

Extracellular vesicles (EVs), which includes exosomes (from the endosomal compartment), microvesicles (formed by budding of

the plasma membrane), and apoptotic bodies (from dying cells), are phosphor lipid vesicles from 30 nm to 1 μm in diameter, used as cargo container by cells to exchange biomolecules such as transmembrane receptors and genetic information [199]. More specifically, their payload can include cytokines, morphogens, MMPs, antigens, DNA, non-coding RNAs, mRNAs, and miRNAs, with the latter being particularly explored in term of the potential functional roles of exosomes [199]. EVs are released by most cell types and have been detected in all bodily fluids. Once released in the extracellular milieu, EVs are taken up by the target cells within the local microenvironment or carried to distant sites though biological fluids [200].

EVs are most likely important regulators of immune cell activity and could therefore modulate tissue repair and regeneration via the immune system (Fig. 3) [201,202]. EVs derived from immune cells have been principally studied in the context of the immune response itself, in particular for cancer immunotherapy [203]. In the context of tissue healing, there are few studies looking into the potential of immune cell-derived EVs. Nevertheless, EVs from immune cells most likely have a role in the crosstalk between immunity and tissue haling [201,202]. On the other hand, the most studied EVs in tissue repair and regeneration have been MSC-derived EVs, whose functions include immunomodulation [204]. For example, MSC-derived EVs have been explored as a treatment for fibrotic liver disease [205]. It was demonstrated that the delivery of exosomes into the liver reduced fibrosis through inhibition of the TGF-β1 signaling pathway, as well as through inhibition of epithelial-to-mesenchymal transition of hepatocytes. This effect was most likely mediated by miR-125b [206]. MSC-derived exosomes can also inhibit macrophage activation by suppressing TLR signaling [207]. In a mouse model of hypoxic pulmonary hypertension, it was shown that the intravenous delivery of MSC-derived exosomes suppresses hypoxic inflammation by inhibition of pro-proliferative pathways via miR-17 [208].

In most of the animal *in vivo* studies, EVs have been delivered by intravenous or intraperitoneal injection every few days [201,202]. Following injection, studies reported very variable half-life for vesicles clearance, ranging from 10 min to 12 h [199]. However, there is currently no evidence that EV administration following tissue injury preferentially homes to the damaged sites [201,202]. To overcome this issue, researchers can use delivery systems to control the release of EVs *in situ*. For example, a commercially available hydrogel (HyStem-HP) has been used to deliver MSC-derived EVs in a rat critical size bone defect model [209]. In addition, EVs can be regarded as natural drug delivery vehicles and be loaded with exogenous therapeutic agents. Since EVs are effective natural systems for polynucleotide (siRNA, miRNA) and protein delivery, one can engineer EVs surface to deliver specific content to specific cell types. For instance, siRNA have been delivered by engineered EVs to suppress pro-inflammatory genes [210]. Targeting was achieved by engineering dendritic cell-derived exosomes to display Lamp2b, an exosomal membrane protein, fused to the neuron-specific RVG peptide and loaded with exogenous siRNA by electroporation. The therapeutic potential of exosome-mediated siRNA delivery was demonstrated in mice, by the significant mRNA and protein knockdown of a therapeutic target in Alzheimer's disease (BACE1). Similar approaches could be used to engineer EVs, in order to promote tissue regeneration via immune modulation.

Overall, EVs are certainly a potential therapeutic for promoting tissue healing via immune modulation. Furthermore, while a recent study has demonstrated that EVs are able to stay in decellularized tissue scaffolds [126], new researches should explore novel methods to deliver EVs and to integrate them into other biomaterial scaffolds.

3.6. Codelivery of immune modulators and morphogens

Because the tissue healing process involves numerous immune and morphogenetic signals operating at the same time or sequentially, the delivery of multiple immune and morphogenetic factors in a spatiotemporal-controlled manner is most likely required to induce an effective regenerative microenvironment. When delivering multiple factors, the first difficulty is to understand which factors should be delivered at which concentration and at what time. Then, the challenge is to develop systems able to deliver the different factors in a spatiotemporally controlled manner.

One interesting approach is to stimulate an M(IFN-γ) macrophage response, which resolves rapidly to transition to a M(IL-4) response [112]. In that regard, Alvarez et al. reviewed multiple strategies to sequentially release different molecules that polarize the response to M(IFN-γ), then re-polarize to M(IL-4), resulting in improved healing [211]. For example, decellularized bones were used as a scaffold and further engineered to sequentially release IFN-γ for inducing a M(IFN-γ) response and IL-4 to transit to a M (IL-4) response [127]. The authors were able to confirm the sequential stimulation of both macrophage phenotypes and observed increased vascularization in functionalized scaffold compared to empty scaffolds in a murine subcutaneous implantation model.

Codelivering growth factors and immune modulators is also a promising approach. For instance, platelet rich plasma (PRP), which contain high level of growth factors, has been delivered in l-lactic acid grafted gelatin with macrophage attracting micelles containing sphingosine-1-phosphate agonist (SEW2871) to increase bone regeneration in mice [212]. Interestingly, SEW2871-micelles and PRP enhanced the level of TNF-α 3 days after application, and increased the anti-inflammatory cytokines IL-10 at day 10. Using the same system, SDF-1 codelivered with SEW2871-micelles was able to more than double the rate of mouse skin wound closure. Most likely, the combination of SDF-1/SEW2871 was able to recruit both MSCs and macrophages to the damaged skin [134]. A similar approach opted for dual delivery of FGF-2 and IL-10 via poly(ethylene argininylaspartate diglyceride (PEAD)/heparin coacervate biomaterial in a mouse myocardial infarct model [173]. The study showed that the combination of FGF-2 and IL-10 is more effective than delivering the growth factor and the cytokine alone. Heart function was restored and fibrosis was significantly reduced. In the context of bone, codelivery of BMP-2 and EP4 agonist in either a PEG nanogel [149] or polylactic acid gel [151] was successful in inducing bone regeneration.

4. Conclusion and future perspectives

A remarkable number of immune cells and immune modulators participate in all phases of the tissue healing process. Therefore controlling the immune system, in particular key immune cell subsets, is a very plausible strategy to promote tissue regeneration. However, the complex mechanisms by which the immune system orchestrates various organs and tissues are still vastly unknown. For instance, while neutrophils are the first circulating immune cells mobilized after tissue injury, their role in the healing process has been somewhat overlooked. They are likely involved in macrophage polarization, although we still do not know exactly in what way. Controlling neutrophil mobilization and functions could be an interesting strategy to promote tissue regeneration.

Macrophages have shown to be critical during most phases of the tissue healing process, but the mechanisms by which they change phenotypes to stimulate tissue repair, fibrosis or full regeneration remain unclear. Thus, further effort is required to understand the contributions of the different macrophage populations

and activation states in multiple organ systems. For instance, inflammatory macrophages mature into anti-inflammatory macrophages in certain type of tissues, while a distinct population of anti-inflammatory macrophages is mobilized in others. Therefore, depending on the tissue or organ targeted, one could develop regenerative strategies aimed at stimulating macrophage polarization or aiming at recruiting pro-wound healing macrophage subsets. The current approaches points towards driving macrophages polarization to M(IL-4)-like phenotypes, using a variety of immune modulators delivered through biomaterials and drug delivery systems. However, one should remember that, although large numbers of M(IFN-γ) macrophages exacerbate tissue injury, persistent activation or sustained recruitment of particular M(IL-4) macrophage subsets contribute to the development of pathological fibrosis. One of the keys could be to reveal the exact mechanisms that drive the expansion of anti-inflammatory/anti-fibrotic macrophages *in vivo* and further implement these mechanisms into regenerative strategies. Perhaps, the answer will come from the situation in neonates, which display macrophage populations with pro-regenerative capacities distinct from the adult. For instance, altering key macrophage transcription factors that stabilize or induce these particular pro-regenerative populations could hold promise to stimulate regeneration.

As with macrophage subsets, there is growing evidence that T cell subsets can have both anti-regenerative and pro-regenerative properties. Yet, the mechanisms driving T cell mobilization, activation and conversion at sites of tissue injury are still largely unknown. We have seen that Tregs are immune-suppressive and particular Treg populations are pro-regenerative. Th2 and γδT cells could also be critical for inducing a pro-regenerative environment. Therefore, biomaterials and the delivery of immunomodulators could be exploited to modulate T cell activities and promote regeneration. One could induce T cell conversion into Tregs from conventional T cells recruited at a site of injury or promote the mobilization of natural Tregs using chemokines. For example, CCR4, the receptor for CCL22 chemokine, is typically expressed more on Tregs compared to conventional T cells [213,214]. One study was able to successfully reduce inflammation and periodontal disease in mice and dogs via injection of CCL22 microparticle powder [214]. Other options could include increasing the amount of anti-inflammatory cytokines typically produced by Tregs, Th2 cells and M(IL-4) macrophages such as IL-4, IL-10 or IL-13.

The aging of the immune system is also a parameter that could be considered when designing regenerative therapies. Indeed, the baseline macrophage polarization states and the activity of T cell subsets may be affected by patient characteristics [42,215]. Along with differences in the number of stem/progenitor cells in neonates and adults, there is now increasing evidence for changes in macrophage phenotypes and T cell activities with age and diseases. Therefore, alterations in the crosstalk between tissue-resident or transplanted stem/progenitor cells and macrophages and/or T cells could have major impacts on tissue regeneration after injury and in aging.

Today, ample evidence suggests that an active control of the immune system is a very plausible therapeutic strategy to induce tissue regeneration. However, because we still have sparse knowledge about the immune mechanisms modulating the tissue healing process, one of the main challenges is to target the right immune cell populations and pathways for the particular tissue or organ that needs to be regenerated. Then, the challenge is to engineer efficient biomaterial and delivery system platforms for controlling the immune-mediated mechanisms of tissue healing. The next generation of regenerative strategies may evolve from typical biomaterial-, stem cell-, or growth factor-centric approaches to an immune-centric approach, seeking to control the immune system as a means of promoting regrowth of tissues and organs.

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References

[1] S.J. Forbes, N. Rosenthal, Preparing the ground for tissue regeneration: from mechanism to therapy, Nat. Med. 20 (2014) 857–869.
[2] S. Hirsiger, H.P. Simmen, C.M. Werner, G.A. Wanner, D. Rittirsch, Danger signals activating the immune response after trauma, Mediators Inflamm. 2012 (2012) 315941.
[3] H. Kono, A. Onda, T. Yanagida, Molecular determinants of sterile inflammation, Curr. Opin. Immunol. 26 (2014) 147–156.
[4] Y. Shi, G. Hu, J. Su, W. Li, Q. Chen, P. Shou, C. Xu, X. Chen, Y. Huang, Z. Zhu, X. Huang, X. Han, N. Xie, G. Ren, Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair, Cell Res. 20 (2010) 510–518.
[5] Y. Shi, J. Su, A.I. Roberts, P. Shou, A.B. Rabson, G. Ren, How mesenchymal stem cells interact with tissue immune responses, Trends Immunol. 33 (2012) 136–143.
[6] Y. Okamura, M. Watari, E.S. Jerud, D.W. Young, S.T. Ishizaka, J. Rose, J.C. Chow, J.F. Strauss, The extra domain A of fibronectin activates Toll-like receptor 4, J. Biol. Chem. 276 (2001) 10229–10233.
[7] T.L. Adair-Kirk, R.M. Senior, Fragments of extracellular matrix as mediators of inflammation, Int. J. Biochem. Cell Biol. 40 (2008) 1101–1110.
[8] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, Nat. Immunol. 11 (2010) 373–384.
[9] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (2010) 805–820.
[10] C.J. Chen, H. Kono, D. Golenbock, G. Reed, S. Akira, K.L. Rock, Identification of a key pathway required for the sterile inflammatory response triggered by dying cells, Nat. Med. 13 (2007) 851–856.
[11] C. Cayrol, J.P. Girard, IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy, Curr. Opin. Immunol. 31 (2014) 31–37.
[12] J. Oyama, C. Blais Jr., X. Liu, M. Pu, L. Kobzik, R.A. Kelly, T. Bourcier, Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4-deficient mice, Circulation 109 (2004) 784–789.
[13] A. Tsung, R. Sahai, H. Tanaka, A. Nakao, M.P. Fink, M.T. Lotze, H. Yang, J. Li, K.J. Tracey, D.A. Geller, T.R. Billiar, The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion, J. Exp. Med. 201 (2005) 1135–1143.
[14] H. Wu, G. Chen, K.R. Wyburn, J. Yin, P. Bertolino, J.M. Eris, S.I. Alexander, A.F. Sharland, S.J. Chadban, TLR4 activation mediates kidney ischemia/reperfusion injury, J. Clin. Invest. 117 (2007) 2847–2859.
[15] S.C. Tang, T.V. Arumugam, X. Xu, A. Cheng, M.R. Mughal, D.G. Jo, J.D. Lathia, D. A. Siler, S. Chigurupati, X. Ouyang, T. Magnus, S. Camandola, M.P. Mattson, Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 13798–13803.
[16] K. Hyakkoku, J. Hamanaka, K. Tsuruma, M. Shimazawa, H. Tanaka, S. Uematsu, S. Akira, N. Inagaki, H. Nagai, H. Hara, Toll-like receptor 4 (TLR4), but not TLR3 or TLR9, knock-out mice have neuroprotective effects against focal cerebral ischemia, Neuroscience 171 (2010) 258–267.
[17] M. Bujak, M. Dobaczewski, K. Chatila, L.H. Mendoza, N. Li, A. Reddy, N.G. Frangogiannis, Interleukin-1 receptor type I signaling critically regulates infarct healing and cardiac remodeling, Am. J. Pathol. 173 (2008) 57–67.
[18] A.A. Thomay, J.M. Daley, E. Sabo, P.J. Worth, L.J. Shelton, M.W. Harty, J.S. Reichner, J.E. Albina, Disruption of interleukin-1 signaling improves the quality of wound healing, Am. J. Pathol. 174 (2009) 2129–2136.
[19] T. Shichita, E. Hasegawa, A. Kimura, R. Morita, R. Sakaguchi, I. Takada, T. Sekiya, H. Ooboshi, T. Kitazono, T. Yanagawa, T. Ishii, H. Takahashi, S. Mori, M. Nishibori, K. Kuroda, S. Akira, K. Miyake, A. Yoshimura, Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain, Nat. Med. 18 (2012) 911–917.
[20] R.E. Mirza, M.M. Fang, W.J. Ennis, T.J. Koh, Blocking interleukin-1beta induces a healing-associated wound macrophage phenotype and improves healing in type 2 diabetes, Diabetes 62 (2013) 2579–2587.
[21] S. Bhattacharyya, Z. Tamaki, W. Wang, M. Hinchcliff, P. Hoover, S. Getsios, E.S. White, J. Varga, FibronectinEDA promotes chronic cutaneous fibrosis through Toll-like receptor signaling, Sci. Transl. Med. 6 (2014). 232ra50.
[22] N. Takemura, T. Kawasaki, J. Kunisawa, S. Sato, A. Lamichhane, K. Kobiyama, T. Aoshi, J. Ito, K. Mizuguchi, T. Karuppuchamy, K. Matsunaga, S. Miyatake, N. Mori, T. Tsujimura, T. Satoh, Y. Kumagai, T. Kawai, D.M. Standley, K.J. Ishii, H. Kiyono, S. Akira, S. Uematsu, Blockade of TLR3 protects mice from lethal radiation-induced gastrointestinal syndrome, Nat. Commun. 5 (2014) 3492.
[23] M.M. Martino, K. Maruyama, G.A. Kuhn, T. Satoh, O. Takeuchi, R. Muller, S. Akira, Inhibition of IL-1R1/MyD88 signalling promotes mesenchymal stem cell-driven tissue regeneration, Nat. Commun. 7 (2016) 11051.
[24] H. Suga, M. Sugaya, H. Fujita, Y. Asano, Y. Tada, T. Kadono, S. Sato, TLR4, rather than TLR2, regulates wound healing through TGF-β and CCL5 expression, J. Dermatol. Sci. 73 (2014) 117–124.
[25] L. Chen, S. Guo, M.J. Ranzer, L.A. DiPietro, Toll-like receptor 4 plays an essential role in early skin wound healing, J. Invest. Dermatol. (2012).
[26] J. Gregorio, S. Meller, C. Conrad, A. Di Nardo, B. Homey, A. Lauerma, N. Arai, R. L. Gallo, J. Digiovanni, M. Gilliet, Plasmacytoid dendritic cells sense skin injury

and promote wound healing through type I interferons, J. Exp. Med. 207 (2010) 2921–2930.
[27] Z. Julier, M.M. Martino, A. De Titta, L. Jeanbart, J.A. Hubbell, The TLR4 agonist fibronectin extra domain a is cryptic, exposed by ELastase-2; use in a fibrin matrix cancer vaccine, Sci. Rep. 5 (2015).
[28] W.R. Jarnagin, D.C. Rockey, V.E. Koteliansky, S.-S. Wang, D.M. Bissell, Expression of variant fibronectins in wound healing: cellular source and biological activity of the EIIIA segment in rat hepatic fibrogenesis, J. Cell Biol. 127 (1994) 2037–2048.
[29] Y.-F. Liao, P.J. Gotwals, V.E. Koteliansky, D. Sheppard, L. Van De Water, The EIIIA segment of fibronectin is a ligand for integrins α9β1 and α4β1 providing a novel mechanism for regulating cell adhesion by alternative splicing, J. Biol. Chem. 277 (2002) 14467–14474.
[30] W.S. To, K.S. Midwood, Plasma and cellular fibronectin: distinct and independent functions during tissue repair, Fibrogenesis Tissue Repair 4 (2011) 21.
[31] A.F. Muro, A.K. Chauhan, S. Gajovic, A. Iaconcig, F. Porro, G. Stanta, F.E. Baralle, Regulated splicing of the fibronectin EDA exon is essential for proper skin wound healing and normal lifespan, J. Cell Biol. 162 (2003) 149–160.
[32] E. Kolaczkowska, P. Kubes, Neutrophil recruitment and function in health and inflammation, Nat. Rev. Immunol. 13 (2013) 159–175.
[33] T.A. Wilgus, S. Roy, J.C. McDaniel, Neutrophils and wound repair: positive actions and negative reactions, Adv Wound Care 2 (2013) 379–388.
[34] R.L. Zemans, N. Briones, M. Campbell, J. McClendon, S.K. Young, T. Suzuki, I.V. Yang, S. De Langhe, S.D. Reynolds, R.J. Mason, M. Kahn, P.M. Henson, S.P. Colgan, G.P. Downey, Neutrophil transmigration triggers repair of the lung epithelium via beta-catenin signaling, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 15990–15995.
[35] V. Brinkmann, U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D.S. Weiss, Y. Weinrauch, A. Zychlinsky, Neutrophil extracellular traps kill bacteria, Science 303 (2004) 1532–1535.
[36] S.L. Wong, M. Demers, K. Martinod, M. Gallant, Y. Wang, A.B. Goldfine, C.R. Kahn, D.D. Wagner, Diabetes primes neutrophils to undergo NETosis, which impairs wound healing, Nat. Med. 21 (2015) 815–819.
[37] J.V. Dovi, L.-K. He, L.A. DiPietro, Accelerated wound closure in neutrophil-depleted mice, J. Leukoc. Biol. 73 (2003) 448–455.
[38] M. Horckmans, L. Ring, J. Duchene, D. Santovito, M.J. Schloss, M. Drechsler, C. Weber, O. Soehnlein, S. Steffens, Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype, Eur. Heart J. (2016). ehw002.
[39] J.M. Schwab, N. Chiang, M. Arita, C.N. Serhan, Resolvin E1 and protectin D1 activate inflammation-resolution programmes, Nature 447 (2007) 869–874.
[40] S.D. Skaper, L. Facci, P. Giusti, Mast cells, glia and neuroinflammation: partners in crime?, Immunology 141 (2014) 314–327
[41] B.C. Wulff, T.A. Wilgus, Mast cell activity in the healing wound: more than meets the eye?, Exp Dermatol. 22 (2013) 507–510.
[42] T.A. Wynn, K.M. Vannella, Macrophages in Tissue Repair, Regeneration, and Fibrosis, Immunity 44 (2016) 450–462.
[43] L.C. Davies, S.J. Jenkins, J.E. Allen, P.R. Taylor, Tissue-resident macrophages, Nat. Immunol. 14 (2013) 986–995.
[44] Y. Okabe, R. Medzhitov, Tissue biology perspective on macrophages, Nat. Immunol. 17 (2016) 9–17.
[45] C. Varol, A. Mildner, S. Jung, Macrophages: development and tissue specialization, Annu. Rev. Immunol. 33 (2015) 643–675.
[46] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound Repair Regener., Nature 453 (2008) 314–321.
[47] M.L. Novak, E.M. Weinheimer-Haus, T.J. Koh, Macrophage activation and skeletal muscle healing following traumatic injury, J. Pathol. 232 (2014) 344–355.
[48] F. Ginhoux, S. Jung, Monocytes and macrophages: developmental pathways and tissue homeostasis, Nat. Rev. Immunol. 14 (2014) 392–404.
[49] C. Auffray, D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Sarnacki, A. Cumano, G. Lauvau, F. Geissmann, Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior, Science 317 (2007) 666–670.
[50] L.M. Carlin, E.G. Stamatiades, C. Auffray, R.N. Hanna, L. Glover, G. Vizcay-Barrena, C.C. Hedrick, H.T. Cook, S. Diebold, F. Geissmann, Nr4a1-dependent Ly6C(low) monocytes monitor endothelial cells and orchestrate their disposal, Cell 153 (2013) 362–375.
[51] F. Geissmann, S. Jung, D.R. Littman, Blood monocytes consist of two principal subsets with distinct migratory properties, Immunity 19 (2003) 71–82.
[52] P. Italiani, D. Boraschi, From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation, Front. Immunol. 5 (2014) 514.
[53] L. Arnold, A. Henry, F. Poron, Y. Baba-Amer, N. van Rooijen, A. Plonquet, R.K. Gherardi, B. Chazaud, Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis, J. Exp. Med. 204 (2007) 1057–1069.
[54] M.J. Crane, J.M. Daley, O. van Houtte, S.K. Brancato, W.L. Henry Jr., J.E. Albina, The monocyte to macrophage transition in the murine sterile wound, PLoS ONE 9 (2014) e86660.
[55] M. Nahrendorf, F.K. Swirski, E. Aikawa, L. Stangenberg, T. Wurdinger, J.L. Figueiredo, P. Libby, R. Weissleder, M.J. Pittet, The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions, J. Exp. Med. 204 (2007) 3037–3047.
[56] Y. Ishida, J.-L. Gao, P.M. Murphy, Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function, J. Immunol. 180 (2008) 569–579.
[57] W. Zhao, H. Lu, X. Wang, R.M. Ransohoff, L. Zhou, CX3CR1 deficiency delays acute skeletal muscle injury repair by impairing macrophage functions, FASEB J. 30 (2016) 380–393.
[58] L. Arnold, H. Perrin, C.B. de Chanville, M. Saclier, P. Hermand, L. Poupel, E. Guyon, F. Licata, W. Carpentier, J. Vilar, CX3CR1 deficiency promotes muscle repair and regeneration by enhancing macrophage ApoE production, Nat. Commun. 6 (2015).
[59] S. Willenborg, T. Lucas, G. van Loo, J.A. Knipper, T. Krieg, I. Haase, B. Brachvogel, M. Hammerschmidt, A. Nagy, N. Ferrara, CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair, Blood 120 (2012) 613–625.
[60] M.L. Novak, T.J. Koh, Macrophage phenotypes during tissue repair, J. Leukoc. Biol. 93 (2013) 875–881.
[61] P.J. Murray, J.E. Allen, S.K. Biswas, E.A. Fisher, D.W. Gilroy, S. Goerdt, S. Gordon, J.A. Hamilton, L.B. Ivashkiv, T. Lawrence, M. Locati, A. Mantovani, F.O. Martinez, J.L. Mege, D.M. Mosser, G. Natoli, J.P. Saeij, J.L. Schultze, K.A. Shirey, A. Sica, J. Suttles, I. Udalova, J.A. van Ginderachter, S.N. Vogel, T.A. Wynn, Macrophage activation and polarization: nomenclature and experimental guidelines, Immunity 41 (2014) 14–20.
[62] K. Essandoh, Y. Li, J. Huo, G.C. Fan, MiRNA-mediated macrophage polarization and its potential role in the regulation of inflammatory response, Shock 46 (2016) 122–131.
[63] X.Q. Wu, Y. Dai, Y. Yang, C. Huang, X.M. Meng, B.M. Wu, J. Li, Emerging role of microRNAs in regulating macrophage activation and polarization in immune response and inflammation, Immunology 148 (2016) 237–248.
[64] L.A. Murray, Q. Chen, M.S. Kramer, D.P. Hesson, R.L. Argentieri, X. Peng, M. Gulati, R.J. Homer, T. Russell, N. van Rooijen, J.A. Elias, C.M. Hogaboam, E.L. Herzog, TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P, Int. J. Biochem. Cell Biol. 43 (2011) 154–162.
[65] J.P. Pradere, J. Kluwe, S. De Minicis, J.J. Jiao, G.Y. Gwak, D.H. Dapito, M.K. Jang, N.D. Guenther, I. Mederacke, R. Friedman, A.C. Dragomir, C. Aloman, R.F. Schwabe, Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice, Hepatology 58 (2013) 1461–1473.
[66] S.K. Madala, J.T. Pesce, T.R. Ramalingam, M.S. Wilson, S. Minnicozzi, A.W. Cheever, R.W. Thompson, M.M. Mentink-Kane, T.A. Wynn, Matrix metalloproteinase 12-deficiency augments extracellular matrix degrading metalloproteinases and attenuates IL-13-dependent fibrosis, J. Immunol. 184 (2010) 3955–3963.
[67] M. Saraiva, A. O'Garra, The regulation of IL-10 production by immune cells, Nat. Rev. Immunol. 10 (2010) 170–181.
[68] P. Soroosh, T.A. Doherty, W. Duan, A.K. Mehta, H. Choi, Y.F. Adams, Z. Mikulski, N. Khorram, P. Rosenthal, D.H. Broide, M. Croft, Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance, J. Exp. Med. 210 (2013) 775–788.
[69] J. Mauer, B. Chaurasia, J. Goldau, M.C. Vogt, J. Ruud, K.D. Nguyen, S. Theurich, A.C. Hausen, J. Schmitz, H.S. Bronneke, E. Estevez, T.L. Allen, A. Mesaros, L. Partridge, M.A. Febbraio, A. Chawla, F.T. Wunderlich, J.C. Bruning, Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin, Nat. Immunol. 15 (2014) 423–430.
[70] J. Pesce, M. Kaviratne, T.R. Ramalingam, R.W. Thompson, J.F. Urban Jr., A.W. Cheever, D.A. Young, M. Collins, M.J. Grusby, T.A. Wynn, The IL-21 receptor augments Th2 effector function and alternative macrophage activation, J. Clin. Invest. 116 (2006) 2044–2055.
[71] J.W. Godwin, A.R. Pinto, N.A. Rosenthal, Macrophages are required for adult salamander limb regeneration, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 9415–9420.
[72] T.A. Petrie, N.S. Strand, C. Tsung-Yang, J.S. Rabinowitz, R.T. Moon, Macrophages modulate adult zebrafish tail fin regeneration, Development 141 (2014) 2581–2591.
[73] A.B. Aurora, E.R. Porrello, W. Tan, A.I. Mahmoud, J.A. Hill, R. Bassel-Duby, H.A. Sadek, E.N. Olson, Macrophages are required for neonatal heart regeneration, J. Clin. Invest. 124 (2014) 1382–1392.
[74] V.E. Miron, A. Boyd, J.W. Zhao, T.J. Yuen, J.M. Ruckh, J.L. Shadrach, P. Van Wijngaarden, A.J. Wagers, A. Williams, R.J. Franklin, C. Ffrench-Constant, M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination, Nat. Neurosci. 16 (2013) 1211–1218.
[75] D. Dal-Secco, J. Wang, Z. Zeng, E. Kolaczkowska, C.H. Wong, B. Petri, R.M. Ransohoff, I.F. Charo, C.N. Jenne, P. Kubes, A dynamic spectrum of monocytes arising from the in situ reprogramming of CCR2+ monocytes at a site of sterile injury, J. Exp. Med. 212 (2015) 447–456.
[76] P. Ramachandran, A. Pellicoro, M.A. Vernon, L. Boulter, R.L. Aucott, A. Ali, S.N. Hartland, V.K. Snowdon, A. Cappon, T.T. Gordon-Walker, M.J. Williams, D.R. Dunbar, J.R. Manning, N. van Rooijen, J.A. Fallowfield, S.J. Forbes, J.P. Iredale, Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) E3186–E3195.
[77] J.A. Stefater, S. Ren, R.A. Lang, J.S. Duffield, Metchnikoff's policemen: macrophages in development, homeostasis and regeneration, Trends Mol. Med. 17 (2011) 743–752.
[78] K. Stark, A. Eckart, S. Haidari, A. Tirniceriu, M. Lorenz, M.L. von Bruhl, F. Gartner, A.G. Khandoga, K.R. Legate, R. Pless, I. Hepper, K. Lauber, B. Walzog, S. Massberg, Capillary and arteriolar pericytes attract innate leukocytes exiting through venules and 'instruct' them with pattern-recognition and motility programs, Nat. Immunol. 14 (2013) 41–51.

[79] A. Armulik, G. Genove, C. Betsholtz, Pericytes: developmental, physiological, and pathological perspectives, problems, and promises, Dev. Cell 21 (2011) 193–215.

[80] A. Dellavalle, G. Maroli, D. Covarello, E. Azzoni, A. Innocenzi, L. Perani, S. Antonini, R. Sambasivan, S. Brunelli, S. Tajbakhsh, G. Cossu, Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells, Nat. Commun. 2 (2011) 499.

[81] R. Katare, F. Riu, K. Mitchell, M. Gubernator, P. Campagnolo, Y. Cui, O. Fortunato, E. Avolio, D. Cesselli, A.P. Beltrami, G. Angelini, C. Emanueli, P. Madeddu, Transplantation of human pericyte progenitor cells improves the repair of infarcted heart through activation of an angiogenic program involving micro-RNA-132, Circ. Res. 109 (2011) 894–906.

[82] A. Pellicoro, P. Ramachandran, J.P. Iredale, J.A. Fallowfield, Liver fibrosis and repair: immune regulation of wound healing in a solid organ, Nat. Rev. Immunol. 14 (2014) 181–194.

[83] M. Vinish, W. Cui, E. Stafford, L. Bae, H. Hawkins, R. Cox, T. Toliver-Kinsky, Dendritic cells modulate burn wound healing by enhancing early proliferation, Wound Repair Regener. (2016).

[84] N. Gao, J. Yin, G.S. Yoon, Q.-S. Mi, X.Y. Fu-Shin, Dendritic cell-epithelium interplay is a determinant factor for corneal epithelial wound repair, Am. J. Pathol. 179 (2011) 2243–2253.

[85] A. Anzai, T. Anzai, S. Nagai, Y. Maekawa, K. Naito, H. Kaneko, Y. Sugano, T. Takahashi, H. Abe, S. Mochizuki, Regulatory role of dendritic cells in postinfarction healing and left ventricular remodeling, Circulation 125 (2012) 1234–1245.

[86] J. Jameson, K. Ugarte, N. Chen, P. Yachi, E. Fuchs, R. Boismenu, W.L. Havran, A role for skin γδ T cells in wound repair, Science 296 (2002) 747–749.

[87] T. Ono, K. Okamoto, T. Nakashima, T. Nitta, S. Hori, Y. Iwakura, H. Takayanagi, IL-17-producing [gamma][delta] T cells enhance bone regeneration, Nat. Commun. 7 (2016).

[88] K. Ramirez, D.A. Witherden, W.L. Havran, All hands on DE (T) C: Epithelial-resident γδ T cells respond to tissue injury, Cell. Immunol. 296 (2015) 57–61.

[89] P.L. Simonian, F. Wehrmann, C.L. Roark, W.K. Born, R.L. O'Brien, A.P. Fontenot, Γ˜δ T cells protect against lung fibrosis via IL-22, J. Exp. Med. 207 (2010) 2239–2253.

[90] S. Reinke, S. Geissler, W. Taylor, K. Schmidt-Bleek, K. Juelke, V. Schwachmeyer, Terminally differentiated CD8 (+) T cells negatively affect bone regeneration in humans, Sci. Transl. Med. 5 (177) (2013) 177ra36, http://dx.doi.org/10.1126/scitranslmed.3004754. Epub 2013/03/22, 5/177/177ra36 [pii], PMID: 23515078.

[91] Y. Liu, L. Wang, T. Kikuiri, K. Akiyama, C. Chen, X. Xu, R. Yang, W. Chen, S. Wang, S. Shi, Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN-[gamma] and TNF-[alpha], Nat. Med. 17 (2011) 1594–1601.

[92] D. Toben, I. Schroeder, T. El Khassawna, M. Mehta, J.-E. Hoffmann, J.-T. Frisch, H. Schell, J. Lienau, A. Serra, A. Radbruch, G.N. Duda, Fracture healing is accelerated in the absence of the adaptive immune system, J. Bone Miner. Res. 26 (2011) 113–124.

[93] A. Nosbaum, N. Prevel, H.-A. Truong, P. Mehta, M. Ettinger, T.C. Scharschmidt, N.H. Ali, M.L. Pauli, A.K. Abbas, M.D. Rosenblum, Cutting edge: regulatory T cells facilitate cutaneous wound healing, J. Immunol. 196 (2016) 2010–2014.

[94] H. Lei, K. Schmidt-Bleek, A. Dienelt, P. Reinke, H.-D. Volk, Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners, Front. Pharmacol. 6 (2015) 184.

[95] G. Trujillo, A.J. Hartigan, C.M. Hogaboam, T regulatory cells and attenuated bleomycin-induced fibrosis in lungs of CCR7-/-mice, Fibrogenesis Tissue Repair 3 (2010) 18.

[96] N.R. Aggarwal, F.R. D'Alessio, K. Tsushima, V.K. Sidhaye, C. Cheadle, D.N. Grigoryev, K.C. Barnes, L.S. King, Regulatory T cell-mediated resolution of lung injury: identification of potential target genes via expression profiling, Physiol. Genomics 41 (2010) 109–119.

[97] B.T. Garibaldi, F.R. D'Alessio, J.R. Mock, D.C. Files, E. Chau, Y. Eto, M.B. Drummond, N.R. Aggarwal, V. Sidhaye, L.S. King, Regulatory T cells reduce acute lung injury fibroproliferation by decreasing fibrocyte recruitment, Am. J. Respir. Cell Mol. Biol. 48 (2013) 35–43.

[98] L.-W. Lai, K.-C. Yong, Y.-H.H. Lien, Pharmacologic recruitment of regulatory T cells as a novel therapy for ischemic acute kidney injury, Kidney Int. 81 (2012) 983–992.

[99] M.T. Gandolfo, H.R. Jang, S.M. Bagnasco, G.-J. Ko, P. Agreda, M.J. Soloski, M.T. Crow, H. Rabb, Mycophenolate mofetil modifies kidney tubular injury and Foxp3+ regulatory T cell trafficking during recovery from experimental ischemia–reperfusion, Transpl. Immunol. 23 (2010) 45–52.

[100] D. Burzyn, W. Kuswanto, D. Kolodin, J.L. Shadrach, M. Cerletti, Y. Jang, E. Sefik, T.G. Tan, A.J. Wagers, C. Benoist, A special population of regulatory T cells potentiates muscle repair, Cell 155 (2013) 1282–1295.

[101] E. Rigamonti, P. Zordan, C. Sciorati, P. Rovere-Querini, S. Brunelli, Macrophage plasticity in skeletal muscle repair, BioMed Res. Int. 2014 (2014).

[102] X. Meng, J. Yang, M. Dong, K. Zhang, E. Tu, Q. Gao, W. Chen, C. Zhang, Y. Zhang, Regulatory T cells in cardiovascular diseases, Nat. Rev. Cardiol. 13 (2016) 167–179.

[103] G. Liu, H. Ma, L. Qiu, L. Li, Y. Cao, J. Ma, Y. Zhao, Phenotypic and functional switch of macrophages induced by regulatory CD4+CD25+ T cells in mice, Immunol. Cell Biol. 89 (2011) 130–142.

[104] M.W. Zaiss Dietmar, J. van Loosdregt, A. Gorlani, P.J. Bekker Cornelis, A. Gröne, M.P. van Bergen en Henegouwen Paul, C. Roovers Rob, J. Coffer Paul, J.A.M. Sijts Alice, Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor, Immunity 38 (2013) 275–284.

[105] N. Arpaia, J.A. Green, B. Moltedo, A. Arvey, S. Hemmers, S. Yuan, P.M. Treuting, A.Y. Rudensky, A distinct function of regulatory T cells in tissue protection, Cell 162 (2015) 1078–1089.

[106] W.L. Havran, J.M. Jameson, Epidermal T cells and wound healing, J. Immunol. 184 (2010) 5423–5428.

[107] L.L. Sharp, J.M. Jameson, G. Cauvi, W.L. Havran, Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1, Nat. Immunol. 6 (2005) 73–79.

[108] A. Toulon, L. Breton, K.R. Taylor, M. Tenenhaus, D. Bhavsar, C. Lanigan, R. Rudolph, J. Jameson, W.L. Havran, A role for human skin–resident T cells in wound healing, J. Exp. Med. 206 (2009) 743–750.

[109] P. Kumar, K. Rajasekaran, J.M. Palmer, M.S. Thakar, S. Malarkannan, IL-22: an evolutionary missing-link authenticating the role of the immune system in tissue regeneration, J. Cancer 4 (2013) 57–65.

[110] J. Kular, J. Tickner, S.M. Chim, J. Xu, An overview of the regulation of bone remodelling at the cellular level, Clin. Biochem. 45 (2012) 863–873.

[111] I. Könnecke, A. Serra, T. El Khassawna, C. Schlundt, H. Schell, A. Hauser, A. Ellinghaus, H.-D. Volk, A. Radbruch, G.N. Duda, T and B cells participate in bone repair by infiltrating the fracture callus in a two-wave fashion, Bone 64 (2014) 155–165.

[112] R. Sridharan, A.R. Cameron, D.J. Kelly, C.J. Kearney, F.J. O'Brien, Biomaterial based modulation of macrophage polarization: a review and suggested design principles, Mater. Today 18 (2015) 313–325.

[113] S. Browne, A. Pandit, Biomaterial-mediated modification of the local inflammatory environment, Front. Bioeng. Biotechnol. 3 (2015) 67.

[114] S.F. Badylak, J.E. Valentin, A.K. Ravindra, G.P. McCabe, A.M. Stewart-Akers, Macrophage phenotype as a determinant of biologic scaffold remodeling, Tissue Eng. Part A 14 (2008) 1835–1842.

[115] S.B. Orenstein, Y. Qiao, U. Klueh, D.L. Kreutzer, Y.W. Novitsky, Activation of human mononuclear cells by porcine biologic meshes in vitro, Hernia: J. Hernias Abdominal Wall Surgery 14 (2010) 401–407.

[116] J.A. Jones, D.T. Chang, H. Meyerson, E. Colton, I.K. Kwon, T. Matsuda, J.M. Anderson, Proteomic analysis and quantification of cytokines and chemokines from biomaterial surface-adherent macrophages and foreign body giant cells, J. Biomed. Mater. Res., Part A 83 (2007) 585–596.

[117] F.Y. McWhorter, T. Wang, P. Nguyen, T. Chung, W.F. Liu, Modulation of macrophage phenotype by cell shape, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 17253–17258.

[118] K. Nakamura, S. Yokohama, M. Yoneda, S. Okamoto, Y. Tamaki, T. Ito, M. Okada, K. Aso, I. Makino, High, but not low, molecular weight hyaluronan prevents T-cell-mediated liver injury by reducing proinflammatory cytokines in mice, J. Gastroenterol. 39 (2004) 346–354.

[119] J.Y. Je, S.K. Kim, Reactive oxygen species scavenging activity of aminoderivatized chitosan with different degree of deacetylation, Bioorg. Med. Chem. 14 (2006) 5989–5994.

[120] N.J. Turner, S.F. Badylak, The use of biologic scaffolds in the treatment of chronic nonhealing wounds, Adv. Wound Care 4 (2015) 490–500.

[121] S.F. Badylak, D.O. Freytes, T.W. Gilbert, Extracellular matrix as a biological scaffold material: structure and function, Acta Biomater. 5 (2009) 1–13.

[122] B.N. Brown, B.D. Ratner, S.B. Goodman, S. Amar, S.F. Badylak, Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine, Biomaterials 33 (2012) 3792–3802.

[123] L. Huleihel, G.S. Hussey, J.D. Naranjo, L. Zhang, J.L. Dziki, N.J. Turner, D.B. Stolz, S.F. Badylak, Matrix-bound nanovesicles within ECM bioscaffolds, Sci. Adv. 2 (2016) e1600502.

[124] A.J. Beattie, T.W. Gilbert, J.P. Guyot, A.J. Yates, S.F. Badylak, Chemoattraction of progenitor cells by remodeling extracellular matrix scaffolds, Tissue Eng. Part A 15 (2008) 1119–1125.

[125] K. Sadtler, K. Estrellas, B.W. Allen, M.T. Wolf, H. Fan, A.J. Tam, C.H. Patel, B.S. Luber, H. Wang, K.R. Wagner, J.D. Powell, F. Housseau, D.M. Pardoll, J.H. Elisseeff, Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells, Science 352 (2016) 366–370.

[126] J.L. Dziki, D.S. Wang, C. Pineda, B.M. Sicari, T. Rausch, S.F. Badylak, Solubilized extracellular matrix bioscaffolds derived from diverse source tissues differentially influence macrophage phenotype, J. Biomed. Mater. Res., Part A 105 (2017) 138–147.

[127] K.L. Spiller, S. Nassiri, C.E. Witherel, R.R. Anfang, J. Ng, K.R. Nakazawa, T. Yu, G. Vunjak-Novakovic, Sequential delivery of immunomodulatory cytokines to facilitate the M1-to-M2 transition of macrophages and enhance vascularization of bone scaffolds, Biomaterials 37 (2015) 194–207.

[128] A. Asea, M. Rehli, E. Kabingu, J.A. Boch, O. Baré, P.E. Auron, M.A. Stevenson, S. K. Calderwood, Novel signal transduction pathway utilized by extracellular HSP70 role of Toll-like receptor (TLR) 2 and TLR4, J. Biol. Chem. 277 (2002) 15028–15034.

[129] J. Kovalchin, R. Wang, M. Wagh, J. Azoulay, M. Sanders, R. Chandawarkar, In vivo delivery of heat shock protein 70 accelerates wound healing by up-regulating macrophage-mediated phagocytosis, Wound Repair Regener. 14 (2006) 129–137.

[130] M. Yamamoto, T. Sato, J. Beren, D. Verthelyi, D.M. Klinman, The acceleration of wound healing in primates by the local administration of immunostimulatory CpG oligonucleotides, Biomaterials 32 (2011) 4238–4242.

[131] M. Kucia, K. Jankowski, R. Reca, M. Wysoczynski, L. Bandura, D.J. Allendorf, J. Zhang, J. Ratajczak, M.Z. Ratajczak, CXCR4–SDF-1 signalling, locomotion, chemotaxis and adhesion, J. Mol. Histol. 35 (2004) 233–245.
[132] T.T. Lau, D.-A. Wang, Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine, Exp. Opin. Biol. Ther. 11 (2011) 189–197.
[133] B.R. Son, L.A. Marquez-Curtis, M. Kucia, M. Wysoczynski, A.R. Turner, J. Ratajczak, M.Z. Ratajczak, A. Janowska-Wieczorek, Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases, Stem Cells 24 (2006) 1254–1264.
[134] Y.H. Kim, Y. Tabata, Recruitment of mesenchymal stem cells and macrophages by dual release of stromal cell-derived factor-1 and a macrophage recruitment agent enhances wound closure, J. Biomed. Mater. Res., Part A 104 (2016) 942–956.
[135] W. Shen, X. Chen, J. Chen, Z. Yin, B.C. Heng, W. Chen, H.-W. Ouyang, The effect of incorporation of exogenous stromal cell-derived factor-1 alpha within a knitted silk-collagen sponge scaffold on tendon regeneration, Biomaterials 31 (2010) 7239–7249.
[136] Y. Kimura, Y. Tabata, Controlled release of stromal-cell-derived factor-1 from gelatin hydrogels enhances angiogenesis, J. Biomater. Sci. Polym. Ed. 21 (2010) 37–51.
[137] S.Y. Rabbany, J. Pastore, M. Yamamoto, T. Miller, S. Rafii, R. Aras, M. Penn, Continuous delivery of stromal cell-derived factor-1 from alginate scaffolds accelerates wound healing, Cell Transplant. 19 (2010) 399–408.
[138] G. Zhang, Y. Nakamura, X. Wang, Q. Hu, L.J. Suggs, J. Zhang, Controlled release of stromal cell-derived factor-1alpha in situ increases c-kit+ cell homing to the infarcted heart, Tissue Eng. 13 (2007) 2063–2071.
[139] P.T. Thevenot, A.M. Nair, J. Shen, P. Lotfi, C.-Y. Ko, L. Tang, The effect of incorporation of SDF-1α into PLGA scaffolds on stem cell recruitment and the inflammatory response, Biomaterials 31 (2010) 3997–4008.
[140] D. Projahn, S. Simsekyilmaz, S. Singh, I. Kanzler, B.K. Kramp, M. Langer, A. Burlacu, J. Bernhagen, D. Klee, A. Zernecke, Controlled intramyocardial release of engineered chemokines by biodegradable hydrogels as a treatment approach of myocardial infarction, J. Cell Mol. Med. 18 (2014) 790–800.
[141] J. Ding, K. Hori, R. Zhang, Y. Marcoux, D. Honardoust, H.A. Shankowsky, E.E. Tredget, Tredget E.E., Stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4 in the formation of postburn hypertrophic scar (HTS), Wound Repair Regener. 19 (2011) 568–578.
[142] K. Noguchi, I. Ishikawa, The roles of cyclooxygenase-2 and prostaglandin E2 in periodontal disease, Periodontology 2000 (43) (2007) 85–101.
[143] L. Shapira, W.A. Soskolne, M. Sela, S. Offenbacher, V. Barak, The secretion of PGE2, IL-1β, IL-6, and TNFα by adherent mononuclear cells from early onset periodontitis patients, J. Periodontol. 65 (1994) 139–146.
[144] V. Paralkar, F. Borovecki, H. Ke, K. Cameron, B. Lefker, W. Grasser, T. Owen, M. Li, P. DaSilva-Jardine, M. Zhou, An EP2 receptor-selective prostaglandin E2 agonist induces bone healing, Proc. Natl. Acad. Sci. 100 (2003) 6736–6740.
[145] S. Namkoong, S.-J. Lee, C.-K. Kim, Y.-M. Kim, H.-T. Chung, H. Lee, J.-A. Han, K.-S. Ha, Y.-G. Kwon, Y.-M. Kim, Prostaglandin E2 stimulates angiogenesis by activating the nitric oxide/cGMP pathway in human umbilical vein endothelial cells, Exp. Mol. Med. 37 (2005) 588–600.
[146] P. Kaliński, C. Hilkens, A. Snijders, F. Snijdewint, M.L. Kapsenberg, IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells, J. Immunol. 159 (1997) 28–35.
[147] L. Cui, S. Yin, W. Liu, N. Li, W. Zhang, Y. Cao, Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2, Tissue Eng. 13 (2007) 1185–1195.
[148] K. Katamura, N. Shintaku, Y. Yamauchi, T. Fukui, Y. Ohshima, M. Mayumi, K. Furusho, Prostaglandin E2 at priming of naive CD4+ T cells inhibits acquisition of ability to produce IFN-gamma and IL-2, but not IL-4 and IL-5, J. Immunol. 155 (1995) 4604–4612.
[149] P. Kamolratanakul, T. Hayata, Y. Ezura, A. Kawamata, C. Hayashi, Y. Yamamoto, H. Hemmi, M. Nagao, R. Hanyu, T. Notomi, Nanogel-based scaffold delivery of prostaglandin E2 receptor–specific agonist in combination with a low dose of growth factor heals critical-size bone defects in mice, Arthritis Rheum. 63 (2011) 1021–1033.
[150] N. Kato, U. Hasegawa, N. Morimoto, Y. Saita, K. Nakashima, Y. Ezura, H. Kurosawa, K. Akiyoshi, M. Noda, Nanogel-based delivery system enhances PGE2 effects on bone formation, J. Cell. Biochem. 101 (2007) 1063–1070.
[151] H. Toyoda, H. Terai, R. Sasaoka, K. Oda, K. Takaoka, Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist, Bone 37 (2005) 555–562.
[152] J.C. Sy, G. Seshadri, S.C. Yang, M. Brown, T. Oh, S. Dikalov, N. Murthy, M.E. Davis, Sustained release of a p38 inhibitor from non-inflammatory microspheres inhibits cardiac dysfunction, Nat. Mater. 7 (2008) 863–868.
[153] C.N. Serhan, Pro-resolving lipid mediators are leads for resolution physiology, Nature 510 (2014) 92–101.
[154] C.N. Serhan, N. Chiang, J. Dalli, The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution, in: Semin. Immunol., Elsevier, 2015, pp. 200–215.
[155] A. Ariel, G. Fredman, Y.-P. Sun, A. Kantarci, T.E. Van Dyke, A.D. Luster, C.N. Serhan, Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression, Nat. Immunol. 7 (2006) 1209–1216.
[156] Y. Tang, M.J. Zhang, J. Hellmann, M. Kosuri, A. Bhatnagar, M. Spite, Proresolution therapy for the treatment of delayed healing of diabetic wounds, Diabetes 62 (2013) 618–627.
[157] S. Hong, H. Tian, Y. Lu, J.M. Laborde, F.A. Muhale, Q. Wang, B.V. Alapure, C.N. Serhan, N.G. Bazan, Neuroprotectin/protectin D1: endogenous biosynthesis and actions on diabetic macrophages in promoting wound healing and innervation impaired by diabetes, Am. J. Physiol. Cell Physiol. 307 (2014) C1058–C1067.
[158] D.P. Vasconcelos, M. Costa, I.F. Amaral, M.A. Barbosa, A.P. Águas, J.N. Barbosa, Modulation of the inflammatory response to chitosan through M2 macrophage polarization using pro-resolution mediators, Biomaterials 37 (2015) 116–123.
[159] D.P. Vasconcelos, A.C. Fonseca, M. Costa, I.F. Amaral, M.A. Barbosa, A.P. Águas, J.N. Barbosa, Macrophage polarization following chitosan implantation, Biomaterials 34 (2013) 9952–9959.
[160] C.P. De Oliveira, N. Magolbo, R. De Aquino, C. Weller, Oral aspirin for treating venous leg ulcers, Cochrane Database Syst. Rev. 2 (2016). CD009432-CD.
[161] I. Cantón, R. Mckean, M. Charnley, K.A. Blackwood, C. Fiorica, A.J. Ryan, S. MacNeil, Development of an Ibuprofen-releasing biodegradable PLA/PGA electrospun scaffold for tissue regeneration, Biotechnol. Bioeng. 105 (2010) 396–408.
[162] L. Varatharajan, A. Thapar, T. Lane, A.B. Munster, A.H. Davies, Pharmacological adjuncts for chronic venous ulcer healing: a systematic review, Phlebology (2015). 0268355515587194.
[163] Q. Wang, H. Li, Y. Xiao, S. Li, B. Li, X. Zhao, L. Ye, B. Guo, X. Chen, Y. Ding, Locally controlled delivery of TNFα antibody from a novel glucose-sensitive scaffold enhances alveolar bone healing in diabetic conditions, J. Control. Release 206 (2015) 232–242.
[164] E.E. Friedrich, L.T. Sun, S. Natesan, D.O. Zamora, R.J. Christy, N.R. Washburn, Effects of hyaluronic acid conjugation on anti-TNF-α inhibition of inflammation in burns, J. Biomed. Mater. Res., Part A 102 (2014) 1527–1536.
[165] Y.S. Kim, H.J. Park, M.H. Hong, P.M. Kang, J.P. Morgan, M.H. Jeong, J.G. Cho, J.C. Park, Y. Ahn, TNF-alpha enhances engraftment of mesenchymal stem cells into infarcted myocardium, Front. Biosci. 14 (2009) 2845–2856.
[166] W. Böcker, D. Docheva, W.C. Prall, V. Egea, E. Pappou, O. Roßmann, C. Popov, W. Mutschler, C. Ries, M. Schieker, IKK-2 is required for TNF-α-induced invasion and proliferation of human mesenchymal stem cells, J. Mol. Med. 86 (2008) 1183–1192.
[167] P.M. Mountziaris, A.G. Mikos, Modulation of the inflammatory response for enhanced bone tissue regeneration, Tissue Eng. Part B: Rev. 14 (2008) 179–186.
[168] C. Yan, W.A. Grimm, W.L. Garner, L. Qin, T. Travis, N. Tan, Y.-P. Han, Epithelial to mesenchymal transition in human skin wound healing is induced by tumor necrosis factor-α through bone morphogenic protein-2, Am. J. Pathol. 176 (2010) 2247–2258.
[169] Y. Ishida, T. Kondo, A. Kimura, K. Matsushima, N. Mukaida, Absence of IL-1 receptor antagonist impaired wound healing along with aberrant NF-κB activation and a reciprocal suppression of TGF-β signal pathway, J. Immunol. 176 (2006) 5598–5606.
[170] J. Chang, Z. Wang, E. Tang, Z. Fan, L. McCauley, R. Franceschi, K. Guan, P.H. Krebsbach, C.-Y. Wang, Inhibition of osteoblastic bone formation by nuclear factor-κB, Nat. Med. 15 (2009) 682–689.
[171] J. Chang, F. Liu, M. Lee, B. Wu, K. Ting, J.N. Zara, C. Soo, Hezaimi K. Al, W. Zou, X. Chen, NF-κB inhibits osteogenic differentiation of mesenchymal stem cells by promoting β-catenin degradation, Proc. Natl. Acad. Sci. 110 (2013) 9469–9474.
[172] A. King, S. Balaji, L.D. Le, T.M. Crombleholme, S.G. Keswani, Regenerative wound healing: the role of interleukin-10, Adv. Wound Care 3 (2014) 315–323.
[173] W.C. Chen, B.G. Lee, D.W. Park, K. Kim, H. Chu, K. Kim, J. Huard, Y. Wang, Controlled dual delivery of fibroblast growth factor-2 and Interleukin-10 by heparin-based coacervate synergistically enhances ischemic heart repair, Biomaterials 72 (2015) 138–151.
[174] N. Mokarram, A. Merchant, V. Mukhatyar, G. Patel, R.V. Bellamkonda, Effect of modulating macrophage phenotype on peripheral nerve repair, Biomaterials 33 (2012) 8793–8801.
[175] A.J. Rao, C. Nich, L.S. Dhulipala, E. Gibon, R. Valladares, S. Zwingenberger, R.L. Smith, S.B. Goodman, Local effect of IL-4 delivery on polyethylene particle induced osteolysis in the murine calvarium, J. Biomed. Mater. Res., Part A 101 (2013) 1926–1934.
[176] J.W. Penn, A.O. Grobbelaar, K.J. Rolfe, The role of the TGF-beta family in wound healing, burns and scarring: a review, Int. J. Burns Trauma 2 (2012) 18–28.
[177] C.J. Johnston, D.J. Smyth, D.W. Dresser, R.M. Maizels, TGF-beta in tolerance, development and regulation of immunity, Cell. Immunol. 299 (2016) 14–22.
[178] M.W. Ferguson, J. Duncan, J. Bond, J. Bush, P. Durani, K. So, L. Taylor, J. Chantrey, T. Mason, G. James, Prophylactic administration of avotermin for improvement of skin scarring: three double-blind, placebo-controlled, phase I/II studies, Lancet 373 (2009) 1264–1274.
[179] P.M. Mountziaris, S.N. Tzouanas, D.C. Sing, P.R. Kramer, F.K. Kasper, A.G. Mikos, Intra-articular controlled release of anti-inflammatory siRNA with biodegradable polymer microparticles ameliorates temporomandibular joint inflammation, Acta Biomater. 8 (2012) 3552–3560.
[180] F. Leuschner, P. Dutta, R. Gorbatov, T.I. Novobrantseva, J.S. Donahoe, G. Courties, K.M. Lee, J.I. Kim, J.F. Markmann, B. Marinelli, P. Panizzi, W.W. Lee, Y. Iwamoto, S. Milstein, H. Epstein-Barash, W. Cantley, J. Wong, V. Cortez-Retamozo, A. Newton, K. Love, P. Libby, M.J. Pittet, F.K. Swirski, V. Koteliansky,

R. Langer, R. Weissleder, D.G. Anderson, M. Nahrendorf, Therapeutic siRNA silencing in inflammatory monocytes in mice, Nat. Biotechnol. 29 (2011) 1005–1010.
[181] H.B. Sager, P. Dutta, J.E. Dahlman, M. Hulsmans, G. Courties, Y. Sun, T. Heidt, C. Vinegoni, A. Borodovsky, K. Fitzgerald, G.R. Wojtkiewicz, Y. Iwamoto, B. Tricot, O.F. Khan, K.J. Kauffman, Y. Xing, T.E. Shaw, P. Libby, R. Langer, R. Weissleder, F. K. Swirski, D.G. Anderson, M. Nahrendorf, RNAi targeting multiple cell adhesion molecules reduces immune cell recruitment and vascular inflammation after myocardial infarction, Sci. Transl. Med. 8 (2016) 342ra80.
[182] H. Yin, R.L. Kanasty, A.A. Eltoukhy, A.J. Vegas, J.R. Dorkin, D.G. Anderson, Non-viral vectors for gene-based therapy, Nat. Rev. Genet. 15 (2014) 541–555.
[183] A. Mehta, D. Baltimore, MicroRNAs as regulatory elements in immune system logic, Nat. Rev. Immunol. 16 (2016) 279–294.
[184] A. Das, M. Sinha, S. Datta, M. Abas, S. Chaffee, C.K. Sen, S. Roy, Monocyte and macrophage plasticity in tissue repair and regeneration, Am. J. Pathol. 185 (2015) 2596–2606.
[185] R.M. O'Connell, D.S. Rao, A.A. Chaudhuri, D. Baltimore, Physiological and pathological roles for microRNAs in the immune system, Nat. Rev. Immunol. 10 (2010) 111–122.
[186] J. Banerjee, Y.C. Chan, C.K. Sen, MicroRNAs in skin and wound healing, Physiol. Genomics 43 (2011) 543–556.
[187] J. Banerjee, C.K. Sen, MicroRNAs in skin and wound healing, MicroRNA Protocols (2013) 343–356.
[188] D. Li, A. Wang, X. Liu, F. Meisgen, J. Grünler, I.R. Botusan, S. Narayanan, E. Erikci, X. Li, L. Blomqvist, MicroRNA-132 enhances transition from inflammation to proliferation during wound healing, J. Clin. Investig. 125 (2015) 3008–3026.
[189] Y. Singh, O.A. Garden, F. Lang, B.S. Cobb, MicroRNA-15b/16 enhances the induction of regulatory T cells by regulating the expression of rictor and mTOR, J. Immunol. 195 (2015) 5667–5677.
[190] D. Baumjohann, K.M. Ansel, MicroRNA-mediated regulation of T helper cell differentiation and plasticity, Nat. Rev. Immunol. 13 (2013) 666–678.
[191] Q.-J. Li, J. Chau, P.J. Ebert, G. Sylvester, H. Min, G. Liu, R. Braich, M. Manoharan, J. Soutschek, P. Skare, MiR-181a is an intrinsic modulator of T cell sensitivity and selection, Cell 129 (2007) 147–161.
[192] A. Sethi, N. Kulkarni, S. Sonar, G. Lal, Role of miRNAs in CD4 T cell plasticity during inflammation and tolerance, Front. Genet. 4 (2013) 8.
[193] J.E. Frith, E.R. Porrello, J.J. Cooper-White, Concise review: new frontiers in microRNA-based tissue regeneration, Stem Cells Transl. Med. 3 (2014) 969–976.
[194] T. Nakasa, M. Ishikawa, M. Shi, H. Shibuya, N. Adachi, M. Ochi, Acceleration of muscle regeneration by local injection of muscle-specific microRNAs in rat skeletal muscle injury model, J. Cell Mol. Med. 14 (2010) 2495–2505.
[195] Y. Zhang, Z. Wang, R.A. Gemeinhart, Progress in microRNA delivery, J. Control. Release 172 (2013) 962–974.
[196] M. Gori, M. Trombetta, D. Santini, A. Rainer, Tissue engineering and microRNAs: future perspectives in regenerative medicine, Exp. Opin. Biol. Ther. 15 (2015) 1601–1622.
[197] J. Li, R. Kooger, M. He, X. Xiao, L. Zheng, Y. Zhang, A supramolecular hydrogel as a carrier to deliver microRNA into the encapsulated cells, Chem. Commun. 50 (2014) 3722–3724.
[198] Y. Morishita, T. Imai, H. Yoshizawa, M. Watanabe, K. Ishibashi, S. Muto, D. Nagata, Delivery of microRNA-146a with polyethylenimine nanoparticles inhibits renal fibrosis in vivo, Int. J. Nanomed. 10 (2015) 3475–3488.
[199] E. van der Pol, A.N. Boing, P. Harrison, A. Sturk, R. Nieuwland, Classification, functions, and clinical relevance of extracellular vesicles, Pharmacol. Rev. 64 (2012) 676–705.
[200] S.E.L. Andaloussi, I. Mager, X.O. Breakefield, M.J. Wood, Extracellular vesicles: biology and emerging therapeutic opportunities, Nat. Rev. Drug Disc. 12 (2013) 347–357.
[201] A.M. Silva, J.H. Teixeira, M.I. Almeida, R.M. Gonçalves, M.A. Barbosa, S.G. Santos, Extracellular vesicles: immunomodulatory messengers in the context of tissue repair/regeneration, Eur. J. Pharm. Sci. (2016).
[202] J.H. Teixeira, A.M. Silva, M.I. Almeida, M.A. Barbosa, S.G. Santos, Circulating extracellular vesicles: their role in tissue repair and regeneration, Transfusion Apheresis Sci. 55 (2016) 53–61.
[203] S. Fais, L. O'Driscoll, F.E. Borras, E. Buzas, G. Camussi, F. Cappello, J. Carvalho, A. Cordeiro da Silva, H. Del Portillo, S. El Andaloussi, Trcek T. Ficko, R. Furlan, A. Hendrix, I. Gursel, V. Kralj-Iglic, B. Kaeffer, M. Kosanovic, M.E. Lekka, G. Lipps, M. Logozzi, A. Marcilla, M. Sammar, A. Llorente, I. Nazarenko, C. Oliveira, G. Pocsfalvi, L. Rajendran, G. Raposo, E. Rohde, P. Siljander, G. van Niel, M.H. Vasconcelos, M. Yanez-Mo, M.L. Yliperttula, N. Zarovni, A.B. Zavec, B. Giebel, Evidence-based clinical use of nanoscale extracellular vesicles in nanomedicine, ACS Nano 10 (2016) 3886–3899.
[204] A. Marote, F.G. Teixeira, B. Mendes-Pinheiro, A.J. Salgado, MSCs-derived exosomes: cell-secreted nanovesicles with regenerative potential, Front. Pharmacol. 7 (2016) 231.
[205] T. Li, Y. Yan, B. Wang, H. Qian, X. Zhang, L. Shen, M. Wang, Y. Zhou, W. Zhu, W. Li, W. Xu, Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis, Stem Cells Dev. 22 (2013) 845–854.
[206] J. Hyun, S. Wang, J. Kim, G.J. Kim, Y. Jung, MicroRNA125b-mediated Hedgehog signaling influences liver regeneration by chorionic plate-derived mesenchymal stem cells, Sci. Rep. 5 (2015) 14135.
[207] D.G. Phinney, M. Di Giuseppe, J. Njah, E. Sala, S. Shiva, C.M. St Croix, D.B. Stolz, S.C. Watkins, Y.P. Di, G.D. Leikauf, J. Kolls, D.W. Riches, G. Deiuliis, N. Kaminski, S.V. Boregowda, D.H. McKenna, L.A. Ortiz, Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs, Nat. Commun. 6 (2015) 8472.
[208] C. Lee, S.A. Mitsialis, M. Aslam, S.H. Vitali, E. Vergadi, G. Konstantinou, K. Sdrimas, A. Fernandez-Gonzalez, S. Kourembanas, Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension, Circulation 126 (2012) 2601–2611.
[209] Y. Qin, L. Wang, Z. Gao, G. Chen, C. Zhang, Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation in vitro and promote bone regeneration in vivo, Sci. Rep. 6 (2016) 21961.
[210] L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal, M.J. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, Nat. Biotechnol. 29 (2011) 341–345.
[211] M.M. Alvarez, J.C. Liu, Santiago G. Trujillo-de, B.-H. Cha, A. Vishwakarma, A. Ghaemmaghami, A. Khademhosseini, Delivery strategies to control inflammatory response: Modulating M1–M2 polarization in tissue engineering applications, J. Control. Release (2016).
[212] Y.-H. Kim, H. Furuya, Y. Tabata, Enhancement of bone regeneration by dual release of a macrophage recruitment agent and platelet-rich plasma from gelatin hydrogels, Biomaterials 35 (2014) 214–224.
[213] A.J. Glowacki, R. Gottardi, S. Yoshizawa, F. Cavalla, G.P. Garlet, C. Sfeir, S.R. Little, Strategies to direct the enrichment, expansion, and recruitment of regulatory cells for the treatment of disease, Ann. Biomed. Eng. 43 (2015) 593–602.
[214] A.J. Glowacki, S. Yoshizawa, S. Jhunjhunwala, A.E. Vieira, G.P. Garlet, C. Sfeir, S.R. Little, Prevention of inflammation-mediated bone loss in murine and canine periodontal disease via recruitment of regulatory lymphocytes, Proc. Natl. Acad. Sci. 110 (2013) 18525–18530.
[215] J. Nikolich-Zugich, Aging of the T cell compartment in mice and humans: from no naive expectations to foggy memories, J. Immunol. 193 (2014) 2622–2629.