surgeries

MDPI

Review

Osteoimmunology: An Overview of the Interplay of the Immune System and the Bone Tissue in Fracture Healing

Rayan Ben Letaifa [1,2,†], Tarek Klaylat [1,2,†], Magdalena Tarchala [1], Chan Gao [3], Prism Schneider [4], Derek H. Rosenzweig [1], Paul A. Martineau [1,2] and Rahul Gawri [1,2,*]

[1] Division of Orthopaedic Surgery, Department of Surgery, Faculty of Medicine, McGill University, Montreal, QC H3A 0E7, Canada; rayan.benletaifa@mail.mcgill.ca (R.B.L.); tarek.klaylat@mail.mcgill.ca (T.K.)
[2] Regenerative Orthopaedics and Innovation Laboratory, Division of Orthopaedic Surgery, Department of Surgery, Faculty of Medicine, McGill University, Montréal, QC H3A 0E7, Canada
[3] Division of Physical Medicine and Rehabilitation, Faculty of Medicine, McGill University, Montréal, QC H3A 0E7, Canada; chan.gao@mcgill.ca
[4] Division of Orthopaedic Surgery, Department of Surgery, Faculty of Medicine, University of Calgary, Calgary, AB T2N 1N4, Canada
* Correspondence: rahul.gawri@mcgill.ca; Tel.: +1-514-934-1934 (ext. 42565)
† These authors contributed equally.

Abstract: Bone healing occurs through three consecutive and interdependent phases. While the acute inflammatory response is vital to fracture healing, chronic and systemic inflammation negatively affect the healing process. The bone tissue relies heavily on the immune system for its normal physiology and turnover. The interactions are more pronounced in injury states, such as fractures and autoimmune disorders. Recently, the field of osteoimmunology, the study of the molecular interplay of the immune and skeletal systems, has gained much-needed attention to develop new therapeutic strategies to accelerate fracture healing and prevent the complications of fracture healing. This review provides an overview of the process of fracture healing and discusses the role of immune cells, their interplay with the released cytokines, and the current state of the art in the field of osteoimmunology.

Keywords: osteoimmunology; fracture repair; non-unions; mast cells; macrophages

Citation: Ben Letaifa, R.; Klaylat, T.; Tarchala, M.; Gao, C.; Schneider, P.; Rosenzweig, D.H.; Martineau, P.A.; Gawri, R. Osteoimmunology: An Overview of the Interplay of the Immune System and the Bone Tissue in Fracture Healing. *Surgeries* **2024**, *5*, 402–414. https://doi.org/10.3390/surgeries5020033

Academic Editor: Cornelis F. M. Sier

Received: 27 September 2023
Revised: 25 April 2024
Accepted: 10 May 2024
Published: 15 May 2024

1. Introduction

Osteoimmunology is a rapidly developing interdisciplinary research field that studies the molecular and cellular interplay of the immune and skeletal systems. The term "osteoimmunology" was first used to describe the relationship between these two systems [1–3]. There has been increasing interest in understanding the interaction of immune regulatory molecules in the skeletal system as poor fracture healing is associated with significant morbidity and a large economic burden on the healthcare system [4,5]. On the popular biomedical research database PubMed, a search from the year 2000 to the present with key search terms "osteoimmunology", "fracture healing immune cells", "bone immune cells", and "fracture immune cells" showed an increase in the number of publications with each passing year, thus indicating the interest this emerging field is garnering (Figure 1).

Uniquely, bone has a high propensity to regenerate and remodel. The optimal result is the achievement of similar architecture and strength compared to its pre-injury state. This is attained by three consecutive and closely orchestrated interdependent phases: inflammation, repair, and remodeling. The initial response to injury triggers a signaling cascade secondary to the inflammatory response. This initial response is critical in the downstream phases: repair and remodeling [6,7]. The first phase of fracture healing is affected by the fracture hematoma's milieu and the fracture repair's biomechanical stability [8]. While it is known that an acute inflammatory response is vital to fracture healing, it has also been shown that chronic and systemic inflammation can have a negative

effect [9–11]. This is seen in patients with polytrauma, sepsis, and autoimmune diseases where fracture healing is defective. Given that the initial immune response to injury plays a critical role in fracture healing, a better understanding of the osteoimmune system in both normal and pathological conditions will help optimize fracture management and allow for directed therapies in the future to improve bone healing. This review will describe the interaction of the immune and skeletal systems during the early fracture healing phase and the downstream molecular cascades that are activated.

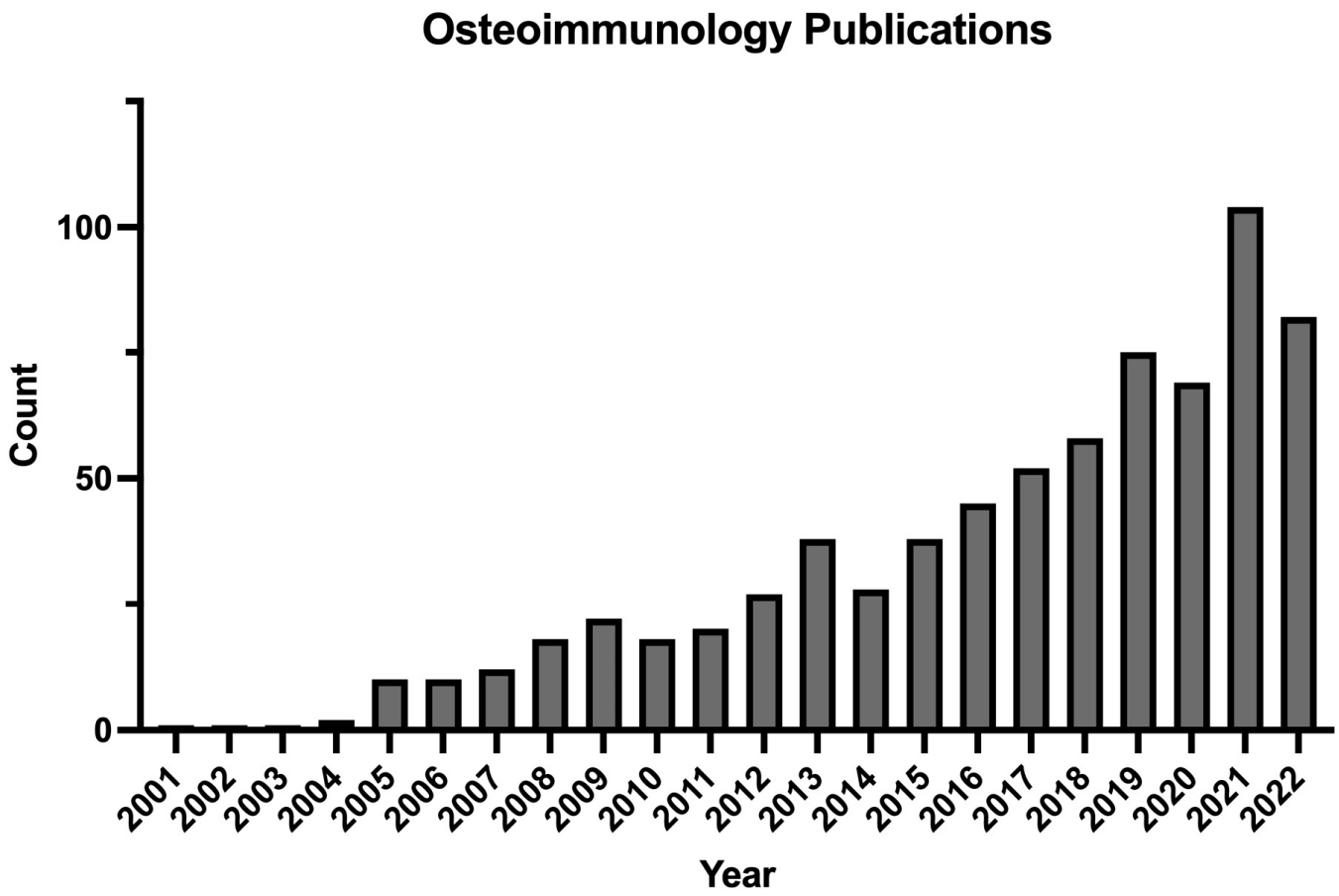

Figure 1. The trend of the increasing number of publications focusing on osteoimmunology indexed on PubMed from the year 2000. This reflects an increasing interest in this emerging field and the research initiatives to advance the field of osteoimmunology.

2. The Normal Inflammatory Cascade and Fracture Healing

The skeletal system and the immune system are inextricably related. This is exhibited by osteoclasts (OCs), which are hematopoietic cells responsible for degrading bone, and osteoblasts (OBs), which are responsible for bone formation. Immune cells, including B cells, T cells, and macrophages, are also derived from hematopoietic stem cells (Figure 2) [12,13]. A vital molecule that is intricately involved in regulating immune responses, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), plays an essential role in differentiating osteoclast precursors (OCPs) into OCs (Figure 2) [14]. In addition to NF-κB, kruppel-like factor 2 (KLF2), a myeloid cell activation and differentiation factor, regulates osteoclast differentiation by modulating autophagy [15]. Other important regulatory chemokines and cytokines of osteoclasts include osteoprotegerin, NF-κB receptor activator ligand (RANKL), and NF-κB receptor (RANK) [16–18]. NF-κB also regulates the immune system by differentiating B and T cells [19]. Further demonstrating the interplay between the immune and skeletal systems, both B and T cells work in concert with OBs to promote the formation of OCs at sites of inflammation, such as at the site of an acute

fracture [3,20–26]. It is evident that local inflammation, mediated by the immune system, plays an important role in the relationship between OBs and OCs and has a vital role during the inflammatory phase of fracture healing.

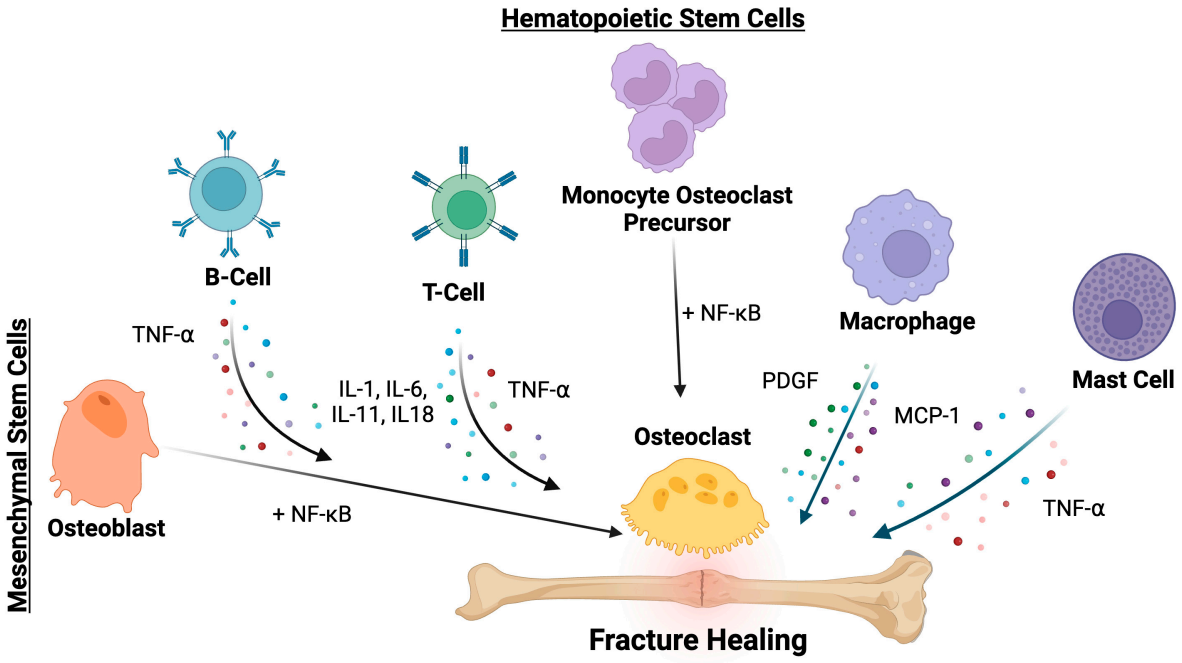

Figure 2. The different cell populations involved in fracture healing are illustrated in the figure. These cells have molecular and cellular crosstalk and regulate each other's recruitment and function at the fracture site to achieve the desired bone healing outcomes. The immune cells release various cytokines and chemokines, such as interleukin 1, 6, 11, and 18 (IL-1, IL-6, IL-11, and IL-18); tumor necrosis factor-alpha (TNF-α); monocyte chemoattractant protein-1 (MCP-1); and platelet-derived growth factor (PDGF), to name a few, to initiate the inflammatory phase of fracture healing, and proceed to the step of bone mineral deposition and calcification of the fracture callus, which is closely followed by fracture remodeling.

3. The Inflammatory Phase of Fracture Healing—The Importance of Fracture Hematoma

Local fracture hematoma is formed by endosteal and surrounding blood vessel disruption during the acute fracture [27]. The acute hematoma is characteristically an acidic and hypoxic environment [28]. This milieu promotes the initiation of a local inflammatory cascade, beginning with the release of pro-inflammatory cytokines and angiogenic factors (Figure 3) [28–32]. A study attempted to implant fracture hematoma components into soft tissue, which resulted in subsequent calcium deposition and bone formation [33]; this suggests that fracture hematoma comprises the required molecular triggers to stimulate bone formation. The fracture hematoma-associated inflammatory cytokines include tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-11 (IL-11), and interleukin-18 (IL-18) [34]. Vascular endothelial growth factor (VEGF) is a vital angiogenic factor in the initial fracture hematoma [35,36]. The ensuing inflammatory response in the surrounding soft tissue leads to vasodilatation and exudes plasma and leukocytes [36–38]. This is followed by local fibrinogen converting to fibrin, which provides a scaffold within the hematoma for recruited inflammatory cells. Furthermore, cellular damage by traumatic fractures releases mitochondrial damage-associated molecular patterns (DAMPs), resulting in the migration of polymorphonuclear neutrophils (PMNs) to the fracture site [39].

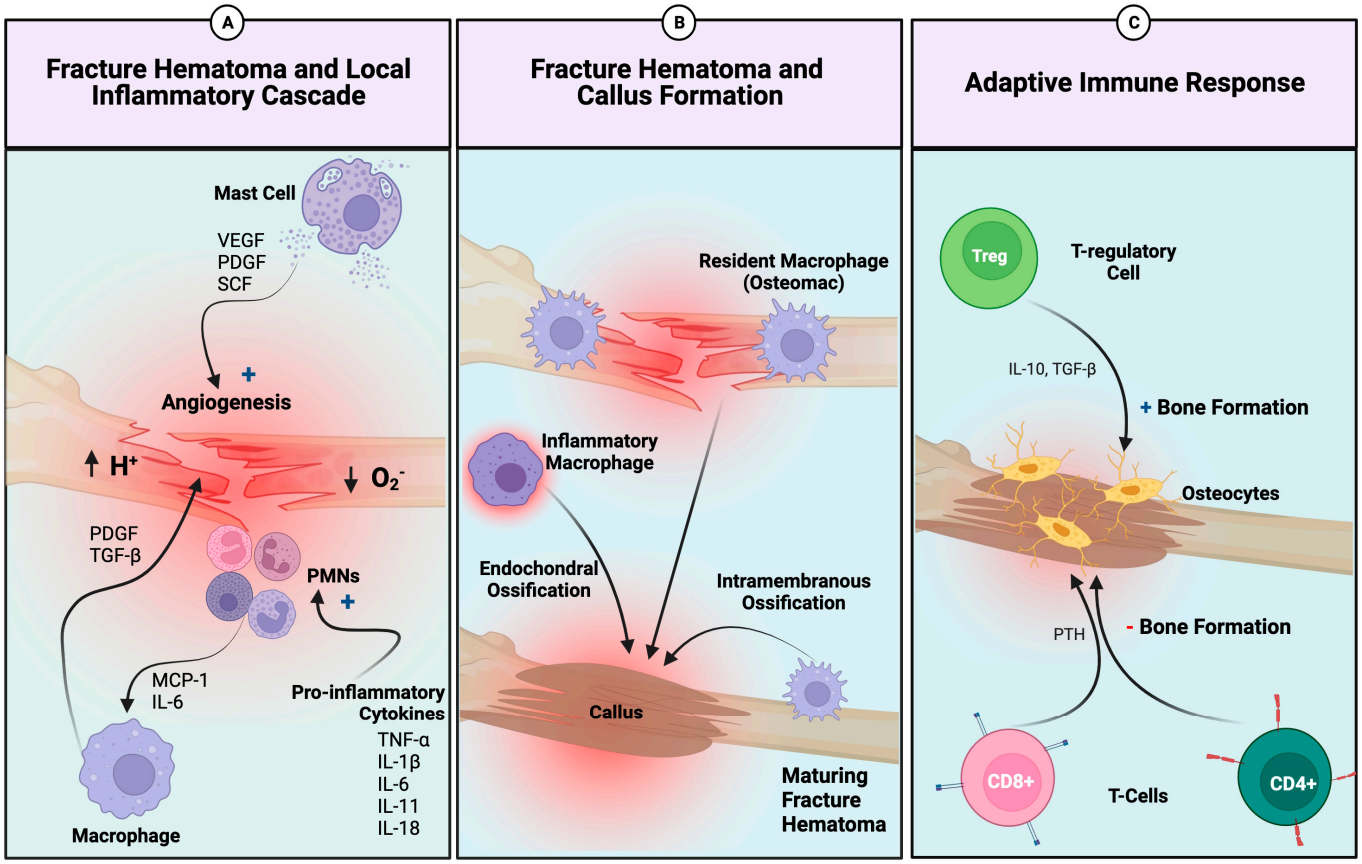

Figure 3. The different stages of fracture healing and the involvement of immune cells at each stage. The process of fracture healing is a tightly orchestrated sequence of the intertwining interplay of cytokines and cellular processes, with specific immune cell functions at play in each stage to create the template for the new bone tissue to form, the newly formed bone tissue to mature, and, later, the newly laid-down bone to remodel.

The leukocyte concentration within the fracture hematoma initially remains similar to the concentration within the peripheral circulation [33]; however, PMNs migrate to the fracture hematoma within the first few hours after injury [40]. Mast cells are leukocytes known for releasing histamine during an allergic reaction, but they also play an important role in the early inflammatory phase of fracture healing. When recruited to the fracture site, mast cells release several factors, including stem cell factor, VEGF, and platelet-derived growth factor (PDGF), which are all important factors for angiogenesis [41–43]. Recent studies of bone healing in mast cell-deficient mice with fractures have demonstrated impaired early neo-vascularization of the fracture callus and deficient bone healing in the absence of mast cells [44]. Contrarily, mast cells were still present at the fracture site at six weeks in normal mice [44]. The study further proposed that mast cells may also play a role in fracture healing beyond the inflammatory phase, exhibiting a bi-phasic role. However, mast cells have also been shown to play their prototypical antagonist role in contributing to compromised bone repair in polytrauma patients [45]; this suggests that mast cells could be a potential target for new treatment options where multiple simultaneous injuries occur.

Leukocyte concentration at the fracture site significantly increases in the first 24 h of injury and helps regulate downstream processes of fracture healing [46]. B cells are another immune cell subtype recruited during the initial inflammatory response, and the absence of this recruitment may result in delayed or non-unions [47]. Studies have also shown that B cells inhibit fracture healing by suppressing osteoblast differentiation by modulating TNF-α and C-C motif chemokine ligand (CCL) 3 [48]. Thus, the regulation of B cell functions through B-regulatory cells (Bregs) is critical during the early fracture

healing phase [47]. Neutrophils, the predominant cell type, remain at the fracture site for only a few hours due to the acidic and anaerobic environment, whereas in typical wound healing, they are present for their lifespan, which is approximately 24 h. During their short presence, neutrophils secrete chemoattractants, such as IL-6 and monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 1 alpha (MIP-1 alpha) to draw cells that can withstand harsh conditions [49–51]. These chemokines attract monocytes, macrophages, and other inflammatory cells [49–52]. As a result, specialized inflammatory cells are sequentially recruited to the acute fracture hematoma in the first few hours post-injury and further potentiate the inflammatory environment.

After the initial recruitment, neutrophils are replaced by longer-living macrophages [53,54]. There are two classes of macrophages, blood-borne inflammatory and tissue-resident macrophages (in this case referred to as osteal macrophages or osteomacs), present on endosteal and periosteal surfaces of neighboring uninjured bone. These macrophages produce transforming growth factor-beta (TGF-β) and PDGF, which have been shown to be involved in fracture healing [54,55]. Specifically, inflammatory macrophages influence endochondral ossification [52], as seen in non-rigid/secondary fracture healing (i.e., casting), whereas osteomacs play an important role in intramembranous bone formation, as seen in rigid/primary fracture healing (i.e., anatomic reduction and rigid fixation) [56]. Osteomacs were required for the deposition of collagen type 1 matrix and bone mineralization in a mouse tibia fracture model [56]. In addition, macrophage polarization influences its extracellular vesicle (EV)-mediated regulatory function. Kang et al. showed that naïve (M0) and M2 polarized macrophage-derived EVs promote bone repair, while M1-derived EVs inhibit bone repair [57]. Furthermore, by compromising inflammatory macrophage recruitment and inflammatory cytokine signaling in animal models of fracture, fracture healing was negatively affected [34,42,52,58,59]. These findings highlight the importance of the initial inflammatory response to injury, which results in macrophage recruitment and modulation of the surrounding immune activity. This allows for the creation of the ideal microenvironment for downstream fracture healing.

During the subsequent stage, T-lymphocytes, another type of immune cell known to withstand the harsh environment of the fracture hematoma, are selectively recruited as part of the adaptive immune response [46,54]. Selective recruitment of T-lymphocytes occurs, while negligible amounts of B-lymphocytes are present at any stage of fracture healing [56]. The crosstalk between the inflammatory response and bone remodeling, with the same cytokines and transcription factors in both processes, is critical to fracture healing. The activation state and cell phenotype of the T cells dictate further downstream fracture healing [60]. For example, T-regulatory cells directly stimulate bone formation through osteocytes, whereas T-CD8+ and T-helper cells can have a negative role on bone healing as they respond primarily to PTH and have been shown to be upregulated in osteoporosis and chronic inflammatory diseases such as rheumatoid arthritis (RA) [60,61].

As illustrated above, the composition of an initial fracture hematoma consists mostly of infiltrated inflammatory cells. The regulated local inflammatory response to injury plays an important role in fracture healing and determines the outcome of the fracture healing [62]. Evacuation of the fracture hematoma disrupts the normal immune and inflammatory response and impairs downstream fracture healing [63–65]. Park et al. serially removed the fracture hematoma in a rabbit open tibia fracture model, resulting in atrophic non-union [64]. Furthermore, Claes et al. measured mRNA expression following osteotomy and mechanically induced delayed fracture healing in a sheep model and showed significantly reduced expression of TGF-β1 and VEGF [66]. Despite this evidence, it remains a common clinical practice to repeatedly irrigate and remove hematoma from the fracture bone site to reduce the risk of infection [67].

Fracture hematoma has many vital components, which should likely be preserved as much as possible during the operative treatment of fractures. This strategy is consistent with the modern treatment of extra-articular diaphyseal fractures, where the objective is to restore length and alignment [68]. Therefore, in many cases, extra-articular diaphyseal

fractures can be treated while preserving the hematoma. On the other hand, when anatomic reduction is the desired outcome, the fracture hematoma is often removed as it may impede the achievement of an accurate reduction [69]. Thus, we should question whether removing the primary hematoma is necessary and whether the secondary hematoma healing potential will be equivalent in function.

Lienau et al. studied the removal of fracture hematomas at four and seven days in a sheep tibial osteotomy model and showed that the new inflammatory response that occurred was concluded to be equivalent to a prolonged inflammatory state [70]. As will be discussed later, prolonged inflammatory states exert a negative effect on fracture healing [70–73]. Furthermore, the secondary hematoma has been shown to have a different microenvironment compared to the initial hematoma [74]. The initial hematoma has an upregulation of osteogenic factors (SPP1 and RUNX2, an osteoblast differentiating transcription factor), whereas the contents of the second hematoma resemble peripheral circulation [28]. Removal of the fracture hematoma also results in a loss of scaffolding and factors that had initially migrated to the fracture site [32]. In addition, the new hematoma that forms does not possess the same factors that are required to synchronize to the current stage of the surrounding healing tissues [32,33].

Therefore, current knowledge suggests that routine fracture hematoma removal should be avoided. In situations where hematoma preservation is not possible, surgeons should weigh the potential advantages of an improved reduction against the adverse effects of disrupting the optimal healing environment.

4. The Importance of Angiogenesis for Osteogenesis

Vascularization is vital for fracture healing. Nonetheless, during the initial few days, the total blood supply to the fracture site is reduced due to periosteal and endosteal vasoconstriction [66,75,76]. For several days following the injury, the fracture hematoma displays osteogenic and angiogenic potential secondary to the cells and factors present due to the initial immune response. Osteoblasts [77], macrophages [78], mast cells [79], and neutrophils [80] are potent producers of VEGF and are believed to be the regulators of angiogenesis in fracture healing [81]. In a rat model of distraction osteogenesis, Sojo et al. showed that VEGF expression was observed only around the newly formed bone. These results further support the hypothesis that angiogenesis is induced by the immune response before osteogenesis [82,83].

The promotion of angiogenesis is critical to fracture healing. Angiopoietins are vascular morphogenetic proteins that help regulate vascular growth by forming large vessels and collateral branches. Specifically, angiopoietin-1 is expressed during the initial inflammatory phase of fracture healing [84]. Furthermore, it has been shown that neutrophils can synthesize angiopoietin 1 [85–87]. Given the early migration of neutrophils to the fracture site, this reiterates the importance of the initial immune response in fracture healing and further suggests that early periosteal vascular in-growth is important for fracture repair and helps to further contribute to downstream angiogenesis. Conversely, conditions such as soft tissue trauma impeding vascularity to the fracture site will interfere with fracture healing. Many animal and human studies have shown this [66,88–90]. The mechanical properties of the callus are also impaired by decreased blood flow [75].

Fracture stability also plays a key role in the early vascular response to injury and revascularization [91,92]. Though optimal fracture stability has yet to be defined, excessive interfragmentary micro-motion has been shown to result in decreased callus revascularization and, in turn, affect downstream osteogenesis [91,93,94]. Therefore, careful soft tissue management and early fracture stability are recommended to optimize early angiogenesis and promote fracture healing.

5. Fracture Healing Impaired by Acute Systemic Inflammation

Polytrauma patients undergo a systemic inflammatory response in the first 24 h after injury [95]. This systemic response has been shown to result in severe osseous clinical

manifestations, such as delayed fracture healing and an increased risk of non-union [1,63]. The unbalanced immune response and increased inflammatory reaction disrupt the fracture healing cascade. As described above, neutrophils are vital in the early inflammatory response. However, the prolonged presence of neutrophils presents a cytotoxic effect. It has been shown that following a major traumatic event, neutrophils remain upregulated and primed for up to 2 weeks [96]. Granulocyte–macrophage colony-stimulating factor (GM-CSF) and TNF-α, both priming agents for neutrophils, have also been shown to be elevated in peripheral blood samples from polytrauma patients [97,98]. Delayed neutrophil apoptosis has also been observed following trauma [96,99], disrupting neutrophil homeostasis and leading to surrounding soft tissue and osseous damage [98]. In addition to elevated systemic inflammation, prolonged neutrophil presence at the site of fracture hematoma has been implicated in delayed fracture healing due to the overstimulation of chondrogenesis and the inhibition of osteogenesis [46].

The negative effect of acute systemic inflammation on fracture healing has been demonstrated in a mouse femoral fracture where a systemic inflammatory state was induced [71]. They showed that only 13% of the mice with systemic inflammation could bridge the fracture, compared to 78% of the controls. Thus, the acute systemic inflammatory response seen in polytrauma plays a significant negative role in fracture healing. Therefore, in the polytrauma setting, one should optimize patient care by minimizing the time to surgery when a second surgical stress response is induced. Limiting the inflammatory response may mitigate negative effects on fracture healing in polytrauma patients.

6. Impaired Fracture Healing Secondary to Chronic Inflammatory States

As previously demonstrated, a local inflammatory response sets in motion a series of specific processes that are critical for fracture healing. However, the maintenance of a prolonged inflammatory state impedes bone repair [100]. In fact, a diminished bone repair capacity is observed in inflammatory diseases, such as diabetes mellitus (DM) and rheumatoid arthritis (RA) [63]. The pathophysiology is believed to be due to an imbalance of immune cells and factors present at the fracture site influencing osteoclastogenesis [60,66]. One pathway is through the activation of NF-κB, which increases osteoclastogenesis [101]. In addition, diabetes itself is an independent risk factor for delayed bone healing [102], and it has also been shown that diabetic patients with a Hemoglobin A1C level greater than 7% have impaired bone healing [103]. Furthermore, the proliferation of macrophages in the bone microenvironment is limited, while the enhanced myeloid differentiation of HSCs results in sustained monocyte recruitment; consequently, macrophages shift towards a pro-inflammatory phenotype, leading to impaired healing [9,104–106]. This often results in fracture healing times being extended by approximately 87% and an increased risk of fracture complications in diabetic patients [107–109]. Decreased expression of RUNX2, an osteoblast differentiating transcription factor, as well as increased IL-8 (a pro-inflammatory cytokine) and the chemotactic receptor CXCR4, can be found in patients with autoimmune diseases [110]. It is thought that an excessive inflammatory response in fracture hematoma in autoimmune diseases may lead to impaired fracture healing. Therefore, optimizing a host's chronic inflammatory disease state could minimize the risk of impaired fracture healing.

7. Cell-Based Therapies and Immunomodulatory Biomaterials for Fracture Healing Applications

Tissue engineering and regenerative medicine approaches have been developed to enhance bone healing, such as using mesenchymal stem cells and various biomaterials. Mesenchymal stem cells constitute an attractive cell source for bone regeneration due to their self-renewal, multipotent, immunomodulatory, and homing properties [111]. The osteogenic potential of such stem cells has been widely studied [112]. In their study, Wang et al. investigated the optimal time to inject bone marrow mesenchymal stem cells (BMSCs) in a murine model of fracture repair [113]. Their study suggests that BMSC injection 7

days post fracture may be an optimal strategy to enhance fracture healing [113]. Moreover, recent studies have focused on stem cell secretomes' regenerative properties and potential in bone tissue engineering applications [114]. Biomaterials have also been investigated for bone regeneration as they provide a 3D structure at the fracture site that supports cell infiltration, adhesion, proliferation, and osteogenic differentiation [115]. Materials such as collagen, hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and polylactic acid attract the most attention in the field of bone tissue engineering for their osteoinductive and osteoconductive properties [115]. Biomaterials and stem cells can also be combined with appropriate bioactive compounds, such as growth factors, to further promote bone healing. Johnson et al. investigated a combination of MSCs with a 3D-printed HA/TCP scaffold in healing critical-sized defects in a swine model [116]. Their results showed that this combination promoted calvarial bone regeneration with mechanical properties and a structure similar to native bone [116]. Many biomaterials have been developed to give them additional immunomodulatory properties by changing the surface topography and chemistry and by immobilizing immunomodulatory ligands [117–120]. Many of these biomaterials have a wide range of applications, primarily in wound healing, and immunomodulatory techniques are now gaining attention to advance the field of osteoimmunology.

8. Osteoimmunological Approaches for Optimal and Accelerated Fracture Healing

Therapeutic immunomodulation has recently attracted significant interest as a viable tissue engineering and regeneration approach that can optimize the host's inflammatory response to accelerate and enhance fracture healing. By enhancing immune cell function or facilitating the identification of neo-antigens, this approach aims to modulate immune cell function to promote repair [121]. In their study, Ramirez-GarciaLuna et al. demonstrate the viability and effects of immunomodulation to modulate bone repair by eliciting a transient and controlled inflammatory response [122]. In this study, the findings show that mice who had a prior subclinical priming fracture surgery performed on one femur showed faster healing rates and enhanced bone remodeling and neo-angiogenesis when a clinical femoral window defect surgery was performed on the contralateral femur compared to mice who only had the clinical femoral window defect surgery [122]. Their work also highlights the importance of macrophages and mast cells in the observed enhanced bone repair [122]. Thus, priming the immune system with subclinical injuries to accelerate the process of full fracture healing is a viable option that harnesses the immune system's close physiological and pathophysiological interactions with the bone tissue.

9. Conclusions

Understanding the interplay between the role of the immune and skeletal systems is imperative to optimize fracture care. This review highlights the role of the acute inflammatory response as a vital step for fracture healing. Conversely, excessive or prolonged acute inflammation and chronic inflammatory states have been shown to impede fracture repair through mechanisms that remain poorly understood and warrant further investigation. To optimize fracture healing, preserving the fracture hematoma, respecting the soft tissue biology, providing early fracture stability, minimizing systemic inflammatory response to injury, and optimizing host chronic inflammatory disease states are recommended. Using a multi-disciplinary approach to target treatment will improve fracture management.

Funding: This work was supported by operating grants awarded to RG by the Start-up funds from the Department of Surgery, McGill University, and by operating grants from the Montreal General Hospital (MGH) Foundation, Le Réseau de recherche en santé buccodentaire et osseuse (RSBO), AO Spine (AOS-Startup-22-050), and the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant-Individual (RGPIN-2023-03826). The figures in this manuscript were made with BioRender after obtaining the required licenses.

Conflicts of Interest: The authors have no financial interests to declare. RG is a scientific advisor for Acorn Biolabs, Canada, and an advisory board member for *JOR-Spine*.

References

1. Targońska, M.; Kochanowska, I.; Ostrowski, K.; Górski, A. Osteoimmunology: New area of research on the associations between the immune and bone systems. *Pol. Arch. Med. Wewn.* **2001**, *105*, 435–440. [PubMed]
2. Arron, J.R.; Choi, Y. Bone versus immune system. *Nature* **2000**, *408*, 535–536. [CrossRef] [PubMed]
3. Nakashima, T.; Takayanagi, H. Osteoimmunology: Crosstalk Between the Immune and Bone Systems. *J. Clin. Immunol.* **2009**, *29*, 555–567. [CrossRef]
4. Aldhafian, M.; Alotaibi, F.; Alzahrani, A.; Almajid, H.; Alamri, A.; Aljandal, A.; Alamri, F.; Alhawas, F.; Khalifa, A.F.M. Patient-dependent factors for fractures union failure among Riyadh population 2016. *J. Fam. Med. Prim. Care* **2020**, *9*, 6224–6227. [CrossRef]
5. Ekegren, C.L.; Edwards, E.R.; De Steiger, R.; Gabbe, B.J. Incidence, Costs and Predictors of Non-Union, Delayed Union and Mal-Union Following Long Bone Fracture. *Int. J. Environ. Res. Public Health* **2018**, *15*, 2845. [CrossRef] [PubMed]
6. Einhorn, T.A.; Gerstenfeld, L.C. Fracture healing: Mechanisms and interventions. *Nat. Rev. Rheumatol.* **2015**, *11*, 45–54. [CrossRef] [PubMed]
7. Marsell, R.; Einhorn, T.A. The biology of fracture healing. *Injury* **2011**, *42*, 551–555. [CrossRef] [PubMed]
8. Foster, A.L.; Moriarty, T.F.; Zalavras, C.; Morgenstern, M.; Jaiprakash, A.; Crawford, R.; Burch, M.-A.; Boot, W.; Tetsworth, K.; Miclau, T.; et al. The influence of biomechanical stability on bone healing and fracture-related infection: The legacy of Stephan Perren. *Injury* **2021**, *52*, 43–52. [CrossRef] [PubMed]
9. Shen, Y.; Zhang, Y.; Zhou, Z.; Wang, J.; Han, D.; Sun, J.; Chen, G.; Tang, Q.; Sun, W.; Chen, L. Dysfunction of macrophages leads to diabetic bone regeneration deficiency. *Front. Immunol.* **2022**, *13*, 990457. [CrossRef]
10. Abou-Khalil, R.; Yang, F.; Mortreux, M.; Lieu, S.; Yu, Y.-Y.; Wurmser, M.; Pereira, C.; Relaix, F.; Miclau, T.; Marcucio, R.S.; et al. Delayed Bone Regeneration Is Linked to Chronic Inflammation in Murine Muscular Dystrophy. *J. Bone Miner. Res.* **2014**, *29*, 304–315. [CrossRef]
11. Goodnough, L.H.; Goodman, S.B. Relationship of Aging, Inflammation, and Skeletal Stem Cells and Their Effects on Fracture Repair. *Curr. Osteoporos. Rep.* **2022**, *20*, 320–325. [CrossRef] [PubMed]
12. Ratajczak, M.Z.; Zuba-Surma, E.K.; Wojakowski, W.; Ratajczak, J.; Kucia, M. Bone Marrow—Home of Versatile Stem Cells. *Transfus. Med. Hemotherapy* **2008**, *35*, 248–259. [CrossRef] [PubMed]
13. Karsenty, G.; Wagner, E.F. Reaching a Genetic and Molecular Understanding of Skeletal Development. *Dev. Cell* **2002**, *2*, 389–406. [CrossRef]
14. Zhang, Y.; Ma, C.; Liu, C.; Wu, W. NF-kappaB promotes osteoclast differentiation by overexpressing MITF via down regulating microRNA-1276 expression. *Life Sci.* **2020**, *258*, 118093. [CrossRef] [PubMed]
15. Laha, D.; Deb, M.; Das, H. KLF2 (kruppel-like factor 2 [lung]) regulates osteoclastogenesis by modulating autophagy. *Autophagy* **2019**, *15*, 2063–2075. [CrossRef]
16. Shiotani, A.; Takami, M.; Itoh, K.; Shibasaki, Y.; Sasaki, T. Regulation of osteoclast differentiation and function by receptor activator of NFkB ligand and osteoprotegerin. *Anat. Rec.* **2002**, *268*, 137–146. [CrossRef] [PubMed]
17. Ono, T.; Hayashi, M.; Sasaki, F.; Nakashima, T. RANKL biology: Bone metabolism, the immune system, and beyond. *Inflamm. Regen.* **2020**, *40*, 2. [CrossRef] [PubMed]
18. Udagawa, N.; Koide, M.; Nakamura, M.; Nakamichi, Y.; Yamashita, T.; Uehara, S.; Kobayashi, Y.; Furuya, Y.; Yasuda, H.; Fukuda, C.; et al. Osteoclast differentiation by RANKL and OPG signaling pathways. *J. Bone Miner. Metab.* **2021**, *39*, 19–26. [CrossRef] [PubMed]
19. Boyce, B.F.; Xing, L.; Franzoso, G.; Siebenlist, U. Requirement for NF-kappaB in osteoclast and B-cell development. *Genes Dev.* **1997**, *11*, 3482–3496.
20. Zhang, W.; Dang, K.; Huai, Y.; Qian, A. Osteoimmunology: The Regulatory Roles of T Lymphocytes in Osteoporosis. *Front. Endocrinol.* **2020**, *11*, 465. [CrossRef]
21. Deng, Z.; Zhang, Q.; Zhao, Z.; Li, Y.; Chen, X.; Lin, Z.; Deng, Z.; Liu, J.; Duan, L.; Wang, D. Crosstalk between immune cells and bone cells or chondrocytes. *Int. Immunopharmacol.* **2021**, *101 Pt A*, 108179. [CrossRef]
22. Croes, M.; Öner, F.C.; van Neerven, D.; Sabir, E.; Kruyt, M.C.; Blokhuis, T.J.; Dhert, W.J.; Alblas, J. Proinflammatory T cells and IL-17 stimulate osteoblast differentiation. *Bone* **2016**, *84*, 262–270. [CrossRef] [PubMed]
23. Du, D.; Zhou, Z.; Zhu, L.; Hu, X.; Lu, J.; Shi, C.; Chen, F.; Chen, A. TNF-alpha suppresses osteogenic differentiation of MSCs by accelerating P2Y(2) receptor in estrogen-deficiency induced osteoporosis. *Bone* **2018**, *117*, 161–170. [CrossRef] [PubMed]
24. Li, Y.; Toraldo, G.; Li, A.; Yang, X.; Zhang, H.; Qian, W.-P.; Weitzmann, M.N. B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass in vivo. *Blood* **2007**, *109*, 3839–3848. [CrossRef] [PubMed]
25. Manabe, N.; Kawaguchi, H.; Chikuda, H.; Miyaura, C.; Inada, M.; Nagai, R.; Nabeshima, Y.-I.; Nakamura, K.; Sinclair, A.M.; Scheuermann, R.H.; et al. Connection Between B Lymphocyte and Osteoclast Differentiation Pathways. *J. Immunol.* **2001**, *167*, 2625–2631. [CrossRef]
26. Nam, D.; Mau, E.; Wang, Y.; Wright, D.; Silkstone, D.; Whetstone, H.; Whyne, C.; Alman, B. T-Lymphocytes Enable Osteoblast Maturation via IL-17F during the Early Phase of Fracture Repair. *PLoS ONE* **2012**, *7*, e40044. [CrossRef]

27. Ma, Y.; Zhou, Y.; Wu, F.; Ji, W.; Zhang, J.; Wang, X. The Bidirectional Interactions Between Inflammation and Coagulation in Fracture Hematoma. *Tissue Eng. Part B Rev.* **2019**, *25*, 46–54. [CrossRef]
28. Kolar, P.; Gaber, T.; Perka, C.; Duda, G.N.; Buttgereit, F. Human Early Fracture Hematoma Is Characterized by Inflammation and Hypoxia. *Clin. Orthop. Relat. Res.* **2011**, *469*, 3118–3126. [CrossRef]
29. Einhorn, T.A.; Majeska, R.J.; Rush, E.B.; Levine, P.M.; Horowitz, M.C. The expression of cytokine activity by fracture callus. *J. Bone Miner. Res.* **1995**, *10*, 1272–1281. [CrossRef]
30. Hoff, P.; Gaber, T.; Strehl, C.; Schmidt-Bleek, K.; Lang, A.; Huscher, D.; Burmester, G.R.; Schmidmaier, G.; Perka, C.; Duda, G.N.; et al. Immunological characterization of the early human fracture hematoma. *Immunol. Res.* **2016**, *64*, 1195–1206. [CrossRef]
31. Walters, G.; Pountos, I.; Giannoudis, P.V. The cytokines and micro-environment of fracture haematoma: Current evidence. *J. Tissue Eng. Regen. Med.* **2018**, *12*, e1662–e1677. [CrossRef] [PubMed]
32. Kolar, P.; Schmidt-Bleek, K.; Schell, H.; Gaber, T.; Toben, D.; Schmidmaier, G.; Perka, C.; Buttgereit, F.; Duda, G.N. The Early Fracture Hematoma and Its Potential Role in Fracture Healing. *Tissue Eng. Part B Rev.* **2010**, *16*, 427–434. [CrossRef] [PubMed]
33. Schmidt-Bleek, K.; Schell, H.; Kolar, P.; Pfaff, M.; Perka, C.; Buttgereit, F.; Duda, G.; Lienau, J. Cellular composition of the initial fracture hematoma compared to a muscle hematoma: A study in sheep. *J. Orthop. Res.* **2009**, *27*, 1147–1151. [CrossRef] [PubMed]
34. Gerstenfeld, L.; Cho, T.-J.; Kon, T.; Aizawa, T.; Tsay, A.; Fitch, J.; Barnes, G.; Graves, D.; Einhorn, T. Impaired Fracture Healing in the Absence of TNF-α Signaling: The Role of TNF-α in Endochondral Cartilage Resorption. *J. Bone Miner. Res.* **2003**, *18*, 1584–1592. [CrossRef] [PubMed]
35. Street, J.; Winter, D.; Wang, J.H.; Wakai, A.; McGuinness, A.; Redmond, H.P. Is human fracture hematoma inherently angiogenic? *Clin. Orthop. Relat. Res.* **2000**, *378*, 224–237. [CrossRef]
36. Lienau, J.; Schmidt-Bleek, K.; Peters, A.; Haschke, F.; Duda, G.N.; Perka, C.; Bail, H.J.; Schütze, N.; Jakob, F.; Schell, H. Differential regulation of blood vessel formation between standard and delayed bone healing. *J. Orthop. Res.* **2009**, *27*, 1133–1140. [CrossRef]
37. Wray, J.B. Acute Changes in Femoral Arterial Blood Flow after Closed Tibial Fracture in Dogs. *J. Bone Jt. Surg.* **1964**, *46*, 1262–1268. [CrossRef]
38. McKibbin, B. The biology of fracture healing in long bones. *J. Bone Jt. Surg.* **1978**, *60*, 150–162. [CrossRef]
39. Zhang, Q.; Raoof, M.; Chen, Y.; Sumi, Y.; Sursal, T.; Junger, W.; Brohi, K.; Itagaki, K.; Hauser, C.J. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* **2010**, *464*, 104–107. [CrossRef]
40. Bastian, O.W.; Koenderman, L.; Alblas, J.; Leenen, L.P.; Blokhuis, T.J. Neutrophils contribute to fracture healing by synthesizing fibronectin + extracellular matrix rapidly after injury. *Clin. Immunol.* **2016**, *164*, 78–84. [CrossRef]
41. Feoktistov, I.; Ryzhov, S.; Goldstein, A.E.; Biaggioni, I. Mast cell-mediated stimulation of angiogenesis: Cooperative interaction between A2B and A3 adenosine receptors. *Circ. Res.* **2003**, *92*, 485–492. [CrossRef]
42. McHale, C.; Mohammed, Z.; Gomez, G. Human Skin-Derived Mast Cells Spontaneously Secrete Several Angiogenesis-Related Factors. *Front. Immunol.* **2019**, *10*, 1445. [CrossRef]
43. Enrico Crivellato, D.R. *Role of Mast Cells in Angiogenesis, in Biochemical Basis and Therapeutic Implications of Angiogenesis*; Springer: Berlin/Heidelberg, Germany, 2013; pp. 107–121.
44. Behrends, D.; Cheng, L.; Sullivan, M.; Wang, M.; Roby, G.; Zayed, N.; Gao, C.; Henderson, J.; Martineau, P. Defective bone repair in mast cell deficient mice with c-Kit loss of function. *Eur. Cells Mater.* **2014**, *28*, 209–221; discussion 221–222. [CrossRef] [PubMed]
45. Ragipoglu, D.; Bülow, J.; Hauff, K.; Voss, M.; Haffner-Luntzer, M.; Dudeck, A.; Ignatius, A.; Fischer, V. Mast Cells Drive Systemic Inflammation and Compromised Bone Repair After Trauma. *Front. Immunol.* **2022**, *13*, 883707. [CrossRef]
46. Bastian, O.; Pillay, J.; Alblas, J.; Leenen, L.; Koenderman, L.; Blokhuis, T. Systemic inflammation and fracture healing. *J. Leukoc. Biol.* **2011**, *89*, 669–673. [CrossRef] [PubMed]
47. Sun, G.; Wang, Y.; Ti, Y.; Wang, J.; Zhao, J.; Qian, H. Regulatory B cell is critical in bone union process through suppressing proinflammatory cytokines and stimulating Foxp3 in Treg cells. *Clin. Exp. Pharmacol. Physiol.* **2017**, *44*, 455–462. [CrossRef] [PubMed]
48. Sun, W.; Meednu, N.; Rosenberg, A.; Rangel-Moreno, J.; Wang, V.; Glanzman, J.; Owen, T.; Zhou, X.; Zhang, H.; Boyce, B.F.; et al. B cells inhibit bone formation in rheumatoid arthritis by suppressing osteoblast differentiation. *Nat. Commun.* **2018**, *9*, 5127. [CrossRef]
49. Chung, R.; Cool, J.C.; Scherer, M.A.; Foster, B.K.; Xian, C.J.; Scherer, M.A. Roles of neutrophil-mediated inflammatory response in the bony repair of injured growth plate cartilage in young rats. *J. Leukoc. Biol.* **2006**, *80*, 1272–1280. [CrossRef]
50. Hurst, S.M.; Wilkinson, T.S.; McLoughlin, R.M.; Jones, S.; Horiuchi, S.; Yamamoto, N.; Rose-John, S.; Fuller, G.M.; Topley, N.; A Jones, S. IL-6 and Its Soluble Receptor Orchestrate a Temporal Switch in the Pattern of Leukocyte Recruitment Seen during Acute Inflammation. *Immunity* **2001**, *14*, 705–714. [CrossRef]
51. Kasama, T.; Strieter, R.M.; Standiford, T.J.; Burdick, M.D.; Kunkel, S.L. Expression and regulation of human neutrophil-derived macrophage inflammatory protein 1 alpha. *J. Exp. Med.* **1993**, *178*, 63–72. [CrossRef]
52. Xing, Z.; Lu, C.; Hu, D.; Yu, Y.-Y.; Wang, X.; Colnot, C.; Nakamura, M.; Wu, Y.; Miclau, T.; Marcucio, R.S. Multiple roles for CCR2 during fracture healing. *Dis. Model. Mech.* **2010**, *3*, 451–458. [CrossRef]
53. Muire, P.J.; Mangum, L.H.; Wenke, J.C. Time Course of Immune Response and Immunomodulation During Normal and Delayed Healing of Musculoskeletal Wounds. *Front. Immunol.* **2020**, *11*, 1056. [CrossRef]
54. Andrew, J.G.; Andrew, S.; Freemont, A.; Marsh, D. Inflammatory cells in normal human fracture healing. *Acta Orthop. Scand.* **1994**, *65*, 462–466. [CrossRef]

55. Baht, G.S.; Vi, L.; Alman, B.A. The Role of the Immune Cells in Fracture Healing. *Curr. Osteoporos. Rep.* **2018**, *16*, 138–145. [CrossRef]
56. Alexander, K.A.; Chang, M.K.; Maylin, E.R.; Kohler, T.; Müller, R.; Wu, A.C.; Van Rooijen, N.; Sweet, M.J.; Hume, D.A.; Raggatt, L.J.; et al. Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. *J. Bone Miner. Res.* **2011**, *26*, 1517–1532. [CrossRef]
57. Kang, M.; Huang, C.-C.; Lu, Y.; Shirazi, S.; Gajendrareddy, P.; Ravindran, S.; Cooper, L.F. Bone regeneration is mediated by macrophage extracellular vesicles. *Bone* **2020**, *141*, 115627. [CrossRef]
58. Gerstenfeld, L.; Cho, T.-J.; Kon, T.; Aizawa, T.; Cruceta, J.; Graves, B.; Einhorn, T. Impaired Intramembranous Bone Formation during Bone Repair in the Absence of Tumor Necrosis Factor-Alpha Signaling. *Cells Tissues Organs* **2001**, *169*, 285–294. [CrossRef]
59. Yang, X.; Ricciardi, B.F.; Hernandez-Soria, A.; Shi, Y.; Camacho, N.P.; Bostrom, M.P. Callus mineralization and maturation are delayed during fracture healing in interleukin-6 knockout mice. *Bone* **2007**, *41*, 928–936. [CrossRef]
60. Ginaldi, L.; De Martinis, M. Osteoimmunology and Beyond. *Curr. Med. Chem.* **2016**, *23*, 3754–3774. [CrossRef]
61. Schlundt, C.; Reinke, S.; Geissler, S.; Bucher, C.H.; Giannini, C.; Märdian, S.; Dahne, M.; Kleber, C.; Samans, B.; Baron, U.; et al. Individual Effector/Regulator T Cell Ratios Impact Bone Regeneration. *Front. Immunol.* **2019**, *10*, 1954. [CrossRef]
62. El-Jawhari, J.J.; Jones, E.; Giannoudis, P.V. The roles of immune cells in bone healing; what we know, do not know and future perspectives. *Injury* **2016**, *47*, 2399–2406. [CrossRef]
63. Claes, L.; Recknagel, S.; Ignatius, A. Fracture healing under healthy and inflammatory conditions. *Nat. Rev. Rheumatol.* **2012**, *8*, 133–143. [CrossRef] [PubMed]
64. Park, S.; Silva, M.; Bahk, W.; McKellop, H.; Lieberman, J.R. Effect of repeated irrigation and debridement on fracture healing in an animal model. *J. Orthop. Res.* **2002**, *20*, 1197–1204. [CrossRef] [PubMed]
65. Mizuno, K.; Mineo, K.; Tachibana, T.; Sumi, M.; Matsubara, T.; Hirohata, K. The osteogenetic potential of fracture haematoma. Subperiosteal and intramuscular transplantation of the haematoma. *J. Bone Jt. Surg. Br.* **1990**, *72*, 822–829. [CrossRef]
66. Claes, L.; Maurer-Klein, N.; Henke, T.; Gerngross, H.; Melnyk, M.; Augat, P. Moderate soft tissue trauma delays new bone formation only in the early phase of fracture healing. *J. Orthop. Res.* **2006**, *24*, 1178–1185. [CrossRef] [PubMed]
67. Shiu, H.T.; Leung, P.C.; Ko, C.H. The roles of cellular and molecular components of a hematoma at early stage of bone healing. *J. Tissue Eng. Regen. Med.* **2018**, *12*, e1911–e1925. [CrossRef] [PubMed]
68. Table of Contents—AO Principles of Fracture Management—Third Edition. Available online: https://pfxm3.aoeducation.org/start.html (accessed on 1 January 2024).
69. Helfet, D.L.; Haas, N.P.; Schatzker, J.; Matter, P.; Moser, R.; Hanson, B. AO philosophy and principles of fracture management-its evolution and evaluation. *J. Bone Jt. Surg. Am.* **2003**, *85*, 1156–1160. [CrossRef]
70. Lienau, J.; Schmidt-Bleek, K.; Peters, A.; Weber, H.; Bail, H.J.; Duda, G.N.; Perka, C.; Schell, H. Insight into the Molecular Pathophysiology of Delayed Bone Healing in a Sheep Model. *Tissue Eng. Part A* **2010**, *16*, 191–199. [CrossRef]
71. Behrends, D.A.; Hui, D.; Gao, C.; Awlia, A.; Al-Saran, Y.; Li, A.; Henderson, J.E.; Martineau, P.A. Defective Bone Repair in C57Bl6 Mice With Acute Systemic Inflammation. *Clin. Orthop. Relat. Res.* **2017**, *475*, 906–916. [CrossRef]
72. Kovach, T.K.; Dighe, A.S.; Lobo, P.I.; Cui, Q. Interactions between MSCs and Immune Cells: Implications for Bone Healing. *J. Immunol. Res.* **2015**, *2015*, 752510. [CrossRef]
73. Schmidt-Bleek, K.; Schell, H.; Schulz, N.; Hoff, P.; Perka, C.; Buttgereit, F.; Volk, H.-D.; Lienau, J.; Duda, G.N. Inflammatory phase of bone healing initiates the regenerative healing cascade. *Cell Tissue Res.* **2012**, *347*, 567–573. [CrossRef] [PubMed]
74. Schell, H.; Duda, G.N.; Peters, A.; Tsitsilonis, S.; Johnson, K.A.; Schmidt-Bleek, K. The haematoma and its role in bone healing. *J. Exp. Orthop.* **2017**, *4*, 5. [CrossRef] [PubMed]
75. Grundnes, O.; Reikerås, O. Blood flow and mechanical properties of healing bone. Femoral osteotomies studied in rats. *Acta Orthop.* **1992**, *63*, 487–491. [CrossRef] [PubMed]
76. Watson, E.C.; Adams, R.H. Biology of Bone: The Vasculature of the Skeletal System. *Cold Spring Harb. Perspect. Med.* **2017**, *8*, a031559. [CrossRef] [PubMed]
77. Ai-Aql, Z.; Alagl, A.; Graves, D.; Gerstenfeld, L.; Einhorn, T. Molecular Mechanisms Controlling Bone Formation during Fracture Healing and Distraction Osteogenesis. *J. Dent. Res.* **2008**, *87*, 107–118. [CrossRef] [PubMed]
78. Bluteau, G.; Julien, M.; Magne, D.; Mallein-Gerin, F.; Weiss, P.; Daculsi, G.; Guicheux, J. VEGF and VEGF receptors are differentially expressed in chondrocytes. *Bone* **2007**, *40*, 568–576. [CrossRef] [PubMed]
79. Komi, D.E.A.; Wöhrl, S.; Bielory, L. Mast Cell Biology at Molecular Level: A Comprehensive Review. *Clin. Rev. Allergy Immunol.* **2020**, *58*, 342–365. [CrossRef] [PubMed]
80. Neagoe, P.E.; Brkovic, A.; Hajjar, F.; Sirois, M.G. Expression and release of angiopoietin-1 from human neutrophils: Intracellular mechanisms. *Growth Factors* **2009**, *27*, 335–344. [CrossRef]
81. Keramaris, N.C.; Calori, G.M.; Nikolaou, V.S.; Schemitsch, E.H.; Giannoudis, P.V. Fracture vascularity and bone healing: A systematic review of the role of VEGF. *Injury* **2008**, *39* (Suppl. 2), S45–S57. [CrossRef]
82. Sojo, K.; Sawaki, Y.; Hattori, H.; Mizutani, H.; Ueda, M. Immunohistochemical study of vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2, -4 (BMP-2, -4) on lengthened rat femurs. *J. Cranio-Maxillofac. Surg.* **2005**, *33*, 238–245. [CrossRef]
83. Diomede, F.; Marconi, G.D.; Fonticoli, L.; Pizzicanella, J.; Merciaro, I.; Bramanti, P.; Mazzon, E.; Trubiani, O. Functional Relationship between Osteogenesis and Angiogenesis in Tissue Regeneration. *Int. J. Mol. Sci.* **2020**, *21*, 3242. [CrossRef] [PubMed]

84. Lehmann, W.; Edgar, C.; Wang, K.; Cho, T.-J.; Barnes, G.; Kakar, S.; Graves, D.; Rueger, J.; Gerstenfeld, L.; Einhorn, T. Tumor necrosis factor alpha (TNF-α) coordinately regulates the expression of specific matrix metalloproteinases (MMPS) and angiogenic factors during fracture healing. *Bone* **2005**, *36*, 300–310. [CrossRef]
85. Lavoie, S.S.; Dumas, E.; Vulesevic, B.; Neagoe, P.-E.; White, M.; Sirois, M.G. Synthesis of Human Neutrophil Extracellular Traps Contributes to Angiopoietin-Mediated In Vitro Proinflammatory and Proangiogenic Activities. *J. Immunol.* **2018**, *200*, 3801–3813. [CrossRef]
86. Poto, R.; Cristinziano, L.; Modestino, L.; de Paulis, A.; Marone, G.; Loffredo, S.; Galdiero, M.R.; Varricchi, G. Neutrophil Extracellular Traps, Angiogenesis and Cancer. *Biomedicines* **2022**, *10*, 431. [CrossRef] [PubMed]
87. Charles, E.; Dumont, B.L.; Bonneau, S.; Neagoe, P.-E.; Villeneuve, L.; Räkel, A.; White, M.; Sirois, M.G. Angiopoietin 1 release from human neutrophils is independent from neutrophil extracellular traps (NETs). *BMC Immunol.* **2021**, *22*, 51. [CrossRef]
88. Utvåg, S.E.; Grundnes, O.; Rindal, D.B.; Reikerås, O. Influence of Extensive Muscle Injury on Fracture Healing in Rat Tibia. *J. Orthop. Trauma* **2003**, *17*, 430–435. [CrossRef]
89. Bhandari, M.; Tornetta, P.; Sprague, S.; Najibi, S.; Petrisor, B.; Griffith, L.; Guyatt, G.H. Predictors of Reoperation Following Operative Management of Fractures of the Tibial Shaft. *J. Orthop. Trauma* **2003**, *17*, 353–361. [CrossRef] [PubMed]
90. Hausman, M.; Schaffler, M.; Majeska, R. Prevention of fracture healing in rats by an inhibitor of angiogenesis. *Bone* **2001**, *29*, 560–564. [CrossRef]
91. Claes, L.; Eckert-Hübner, K.; Augat, P. The effect of mechanical stability on local vascularization and tissue differentiation in callus healing. *J. Orthop. Res.* **2002**, *20*, 1099–1105. [CrossRef]
92. Wallace, A.L.M.; Draper, E.R.C.B.; Strachan, R.K.F.; Mccarthy, I.D.; Hughes, S.P.F.M. The Vascular Response to Fracture Micromovement. *Clin. Orthop. Relat. Res.* **1994**, *301*, 281–290. [CrossRef]
93. Simon, U.; Augat, P.; Utz, M.; Claes, L. A numerical model of the fracture healing process that describes tissue development and revascularisation. *Comput. Methods Biomech. Biomed. Eng.* **2011**, *14*, 79–93. [CrossRef]
94. Claes, L.; Eckert-Hübner, K.; Augat, P. The fracture gap size influences the local vascularization and tissue differentiation in callus healing. *Langenbeck's Arch. Surg.* **2003**, *388*, 316–322. [CrossRef] [PubMed]
95. Lord, J.M.; Midwinter, M.J.; Chen, Y.-F.; Belli, A.; Brohi, K.; Kovacs, E.J.; Koenderman, L.; Kubes, P.; Lilford, R.J. The systemic immune response to trauma: An overview of pathophysiology and treatment. *Lancet* **2014**, *384*, 1455–1465. [CrossRef]
96. Ogura, H.; Tanaka, H.; Koh, T.; Hashiguchi, N.; Kuwagata, Y.; Hosotsubo, H.; Shimazu, T.; Sugimoto, H. Priming, Second-Hit Priming, and Apoptosis in Leukocytes from Trauma Patients. *J. Trauma* **1999**, *46*, 774–783; discussion 781-3. [CrossRef]
97. Watanabe, S.; Alexander, M.; Misharin, A.V.; Budinger, G.S. The role of macrophages in the resolution of inflammation. *J. Clin. Investig.* **2019**, *129*, 2619–2628. [CrossRef]
98. Hietbrink, F.; Koenderman, L.; Rijkers, G.; Leenen, L. Trauma: The role of the innate immune system. *World J. Emerg. Surg.* **2006**, *1*, 15. [CrossRef] [PubMed]
99. Biffl, W.L.; West, K.E.; Moore, E.E.; Gonzalez, R.J.; Carnaggio, R.; Offner, P.J.; Silliman, C.C. Neutrophil Apoptosis Is Delayed by Trauma Patients' Plasma via a Mechanism Involving Proinflammatory Phospholipids and Protein Kinase C. *Surg. Infect.* **2001**, *2*, 289–293; discussion 294-5. [CrossRef] [PubMed]
100. Hurtgen, B.J.; Ward, C.L.; Garg, K.; Pollot, B.E.; Goldman, S.M.; McKinley, T.O.; Wenke, J.C.; Corona, B.T. Severe muscle trauma triggers heightened and prolonged local musculoskeletal inflammation and impairs adjacent tibia fracture healing. *J. Musculoskelet. Neuronal Interact.* **2016**, *16*, 122–134. [PubMed]
101. Tak, P.P.; Firestein, G.S. NF-kappaB: A key role in inflammatory diseases. *J. Clin. Investig.* **2001**, *107*, 7–11. [CrossRef]
102. Hernandez, R.K.; Do, T.P.; Critchlow, C.W.; Dent, R.E.; Jick, S.S. Patient-related risk factors for fracture-healing complications in the United Kingdom General Practice Research Database. *Acta Orthop.* **2012**, *83*, 653–660. [CrossRef]
103. Shibuya, N.; Humphers, J.M.; Fluhman, B.L.; Jupiter, D.C. Factors Associated with Nonunion, Delayed Union, and Malunion in Foot and Ankle Surgery in Diabetic Patients. *J. Foot Ankle Surg.* **2013**, *52*, 207–211. [CrossRef] [PubMed]
104. Galvan-Pena, S.; O'Neill, L.A. Metabolic reprograming in macrophage polarization. *Front. Immunol.* **2014**, *5*, 420. [PubMed]
105. Jiao, H.; Xiao, E.; Graves, D.T. Diabetes and Its Effect on Bone and Fracture Healing. *Curr. Osteoporos. Rep.* **2015**, *13*, 327–335. [CrossRef] [PubMed]
106. Vannella, K.M.; Wynn, T.A. Mechanisms of Organ Injury and Repair by Macrophages. *Annu. Rev. Physiol.* **2017**, *79*, 593–617. [CrossRef] [PubMed]
107. Li, G.; Prior, J.C.; Leslie, W.D.; Thabane, L.; Papaioannou, A.; Josse, R.G.; Kaiser, S.M.; Kovacs, C.S.; Anastassiades, T.; Towheed, T.; et al. Frailty and Risk of Fractures in Patients With Type 2 Diabetes. *Diabetes Care* **2019**, *42*, 507–513. [CrossRef] [PubMed]
108. Massera, D.; Biggs, M.L.; Walker, M.D.; Mukamal, K.J.; Ix, J.H.; Djousse, L.; Valderrábano, R.J.; Siscovick, D.S.; Tracy, R.P.; Xue, X.; et al. Biochemical Markers of Bone Turnover and Risk of Incident Diabetes in Older Women: The Cardiovascular Health Study. *Diabetes Care* **2018**, *41*, 1901–1908. [CrossRef] [PubMed]
109. Stabley, J.N.; Prisby, R.D.; Behnke, B.J.; Delp, M.D. Type 2 diabetes alters bone and marrow blood flow and vascular control mechanisms in the ZDF rat. *J. Endocrinol.* **2015**, *225*, 47–58. [CrossRef] [PubMed]
110. Hoff, P.; Gaber, T.; Schmidt-Bleek, K.; Sentürk, U.; Tran, C.L.; Blankenstein, K.; Lütkecosmann, S.; Bredahl, J.; Schüler, H.J.; Simon, P.; et al. Immunologically restricted patients exhibit a pronounced inflammation and inadequate response to hypoxia in fracture hematomas. *Immunol. Res.* **2011**, *51*, 116–122. [CrossRef]

111. Iaquinta, M.R.; Mazzoni, E.; Bononi, I.; Rotondo, J.C.; Mazziotta, C.; Montesi, M.; Sprio, S.; Tampieri, A.; Tognon, M.; Martini, F. Adult Stem Cells for Bone Regeneration and Repair. *Front. Cell Dev. Biol.* **2019**, *7*, 268. [CrossRef]
112. Shen, C.; Yang, C.; Xu, S.; Zhao, H. Comparison of osteogenic differentiation capacity in mesenchymal stem cells derived from human amniotic membrane (AM), umbilical cord (UC), chorionic membrane (CM), and decidua (DC). *Cell Biosci.* **2019**, *9*, 17. [CrossRef]
113. Wang, X.; Wang, C.; Gou, W.; Xu, X.; Wang, Y.; Wang, A.; Xu, W.; Guo, Q.; Liu, S.; Lu, Q.; et al. The optimal time to inject bone mesenchymal stem cells for fracture healing in a murine model. *Stem Cell Res. Ther.* **2018**, *9*, 272. [CrossRef] [PubMed]
114. Zhang, Y.; Xie, Y.; Hao, Z.; Zhou, P.; Wang, P.; Fang, S.; Li, L.; Xu, S.; Xia, Y. Correction to "Umbilical Mesenchymal Stem Cell-Derived Exosome-Encapsulated Hydrogels Accelerate Bone Repair by Enhancing Angiogenesis". *ACS Appl. Mater. Interfaces* **2022**, *14*, 14834–14835. [CrossRef] [PubMed]
115. Stamnitz, S.; Klimczak, A. Mesenchymal Stem Cells, Bioactive Factors, and Scaffolds in Bone Repair: From Research Perspectives to Clinical Practice. *Cells* **2021**, *10*, 1925. [CrossRef] [PubMed]
116. Johnson, Z.M.; Yuan, Y.; Li, X.; Jashashvili, T.; Jamieson, M.; Urata, M.; Chen, Y.; Chai, Y. Mesenchymal Stem Cells and Three-Dimensional-Osteoconductive Scaffold Regenerate Calvarial Bone in Critical Size Defects in Swine. *Stem Cells Transl. Med.* **2021**, *10*, 1170–1183. [CrossRef] [PubMed]
117. Headen, D.M.; Woodward, K.B.; Coronel, M.M.; Shrestha, P.; Weaver, J.D.; Zhao, H.; Tan, M.; Hunckler, M.D.; Bowen, W.S.; Johnson, C.T.; et al. Local immunomodulation with Fas ligand-engineered biomaterials achieves allogeneic islet graft acceptance. *Nat. Mater.* **2018**, *17*, 732–739. [CrossRef] [PubMed]
118. Anderson, A.E.; Wu, I.; Parrillo, A.J.; Wolf, M.T.; Maestas, D.R.; Graham, I.; Tam, A.J.; Payne, R.M.; Aston, J.; Cooney, C.M.; et al. An immunologically active, adipose-derived extracellular matrix biomaterial for soft tissue reconstruction: Concept to clinical trial. *NPJ Regen. Med.* **2022**, *7*, 6. [CrossRef] [PubMed]
119. Witherel, C.E.; Graney, P.L.; Freytes, D.O.; Weingarten, M.S.; Spiller, K.L. Response of human macrophages to wound matrices in vitro. *Wound Repair Regen.* **2016**, *24*, 514–524. [CrossRef] [PubMed]
120. Lee, J.; Byun, H.; Perikamana, S.K.M.; Lee, S.; Shin, H. Current Advances in Immunomodulatory Biomaterials for Bone Regeneration. *Adv. Health Mater.* **2019**, *8*, e1801106. [CrossRef]
121. Mansour, A.; Abu-Nada, L.; Al-Waeli, H.; Mezour, M.A.; Abdallah, M.-N.; Kinsella, J.M.; Kort-Mascort, J.; Henderson, J.E.; Ramirez-Garcialuna, J.L.; Tran, S.D.; et al. Bone extracts immunomodulate and enhance the regenerative performance of dicalcium phosphates bioceramics. *Acta Biomater.* **2019**, *89*, 343–358. [CrossRef]
122. Ramirez-GarciaLuna, J.L.; Rangel-Berridi, K.; Olasubulumi, O.-O.; Rosenzweig, D.H.; Henderson, J.E.; Gawri, R.; Martineau, P.A. Enhanced Bone Remodeling After Fracture Priming. *Calcif. Tissue Int.* **2022**, *110*, 349–366. [CrossRef]