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Review

Insights into Bioengineering Approaches for Aging Bone Regeneration: Strategies to Target Osteoimmunosenescence

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Abstract: The global accumulation of ageing population is a serious problem causing significant health and social burdens. Especially, aging results in reduced bone regeneration potential and increased risk of morbidities and mortality, which calls the urgent need for advanced therapeutic approaches to improve bone regeneration in the aged patients. The aging associated poor bone regeneration capacity can be attributed to the low-grade, sterile chronic inflammation termed "inflammaging", which result in detrimental environment for bone healing. The pathogenesis of inflammaging is mainly due to the senescence of immune cells. The senescent immune cells, especially senescent macrophages play a major role in inflammaging via an inflammatory secretome (senescence-associated secretory phenotype/SASP) which is due to ROS accumulation associated mitochondrial dysfunction, energy metabolism change, decline in oxidized nicotinamide adenine dinucleotide (NAD^+) level and insufficient autophagy. In addition, the SASP can turn the local young cells into senescent cells, a paracrine senescence effect to facilitate senescent cell accumulation and inflammation, which can also be attributed to the insufficient clearance of senescent cells due to phagocytosis deficiency in senescent immune cells. Therefore, in aging bone environment, the interplay between immune and skeletal cells, termed "osteoimmunosenescence" in

this review, not only generates a long-term chronical inflammatory environment to reduce osteogenesis, but also induces senescence in young skeletal progenitor cells to dampen their osteogenic differentiation potential, suggesting osteoimmunosenescence should be considered as a key modulatory target for bone regeneration biomaterials design for the aged patients. In this review, the pathogenesis of inflammaging and the potential impact of osteoimmunosenescence on bone regeneration have been discussed. In addition, to target osteoimmunosenescence, two potential strategies are considered, one is advanced immunomodulation to correct the inflammaging environment, the other is to target immunosenescence, and the current and potential material approaches regarding these two are summarized in this review. Furthermore, it proposes potential strategies to design osteoimmunosenescence-modulating materials by targeting the molecular intersection between senescence and inflammation and by flexibly correct the local environment and environmental responsively induce osteogenesis.

Keywords: aging; immunosenescence; inflammaging; bone regeneration; osteoimmunomodulation; biomaterials; drug delivery; surface property

1. Introduction

The increasing size of the aging population has become a significant global health and social issue. According to the World Health Organization (WHO), one in seven people, or one billion people, were aged 60+ in 2020, and this number is expected to double by 2050. This will cause substantial medical, social, and economic burdens due to associated functional decline and chronic disability globally [1]. Aging is broadly defined as the time-dependent function decline, the preeminent risk factor for the leading cause of morbidity and mortality worldwide [2]. Aging can contribute to the pathogenesis of multiple chronic diseases and geriatric syndromes termed as age-related disease [3]. Aged patients account for over 50% of the total fracture burden [4,5] due to age-associated osteoporosis [6], and decreased bone mass/strength. More importantly, bone healing becomes problematic for the aged patients, resulting in an increased risk of delayed bone union or even non-union, developing a disability or even death [7]. Hip fractures are reported to result in the death of 1 in 3 older adults (50+ years), often within only one year of injury. This mortality is even higher than that of breast cancer in older women [8,9]. Therefore, there is a significant clinical need that needs to be resolved, though current treatments using bone grafts and tissue engineering approaches have not been successful as they are unable to induce satisfactory new bone formation. Even using osteoinductive proteins such as bone morphology protein 2 (BMP-2), which has demonstrated efficacy in clinical bone regeneration, has been unsuccessful in the elderly [10]. Such a situation suggests that, to fulfill the drastically growing clinical needs in the following decades, it is necessary to develop tissue engineering approach to target aging bone healing.

Physiologically, bone regeneration is highly associated with immune system. This interaction between the immune and skeletal systems, termed as osteoimmunology, plays a determinant role in bone regeneration, that a suitable immune microenvironment is required to ensure the osteogenesis process. However, aging bone healing, a detrimental immune microenvironment is developed, as characterized by a low-grade, sterile chronic inflammation termed “inflammaging” [11,12]. The notion of inflammaging was initially introduced by Prof. Franceschi and colleagues in their ground-breaking study in 2000 [13]. The term inflammaging has been proposed to describe the age-related dynamic immune system alterations that lead to a sterile, chronic, low-grade inflammatory state in the absence of overt infection and contributed to the increased prevalence of non-communicable diseases such as cardiovascular disease, diabetes, obesity, cancer and osteoarthritis, in older adults [14]. Inflammaging is characterized by a modest increase in circulating pro-inflammatory factors such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) [15]. Although “inflammation is a beneficial process, designed to contain and eradicate threats to the host organism”, prolonged conditions of low-grade inflammation continuously suppress the resolution of inflammation and cause repeated progression of tissue damage and repair, which eventually results in irreversible tissue remodeling and dysfunction [16], a condition impedes bone healing by impairing the osteogenic differentiation of osteoblast progenitors [17]. More importantly, inflammaging can generate a “senescent environment” to induce the senescence of the young cells [18], which forming a vicious positive feed-back to facilitate the accumulation of both senescent immune and skeletal cells. Hence, in aging bone, the interplay between senescent immune and skeletal cells, termed “osteoimmunosenescence” in this review, is considered as a major contributor and therapeutic target for the impaired bone healing in the elderly.

The cellular interactions involved in the development of inflammaging and the associated microenvironmental changes remain to be determined, however, monocyte-macrophage lineage cells in the innate immune system are considered the key effector cells in chronic inflammatory processes during the pathogenesis of age-associated disease [19]. Recent insights have suggested that extent aging-related intrinsic changes in macrophages, triggered by chronical age-related stimulations, plays a role in inflammaging at an organismal level [20]. During early-stage bone fracture healing, macrophages are the most prevalent immune cells at the healing site (as evidenced by the significantly induced number of infiltrated macrophages in 3 days after injury [21]), which are considered as vital players in osteoimmunology to determine the bone healing process [22]. Macrophages consist of three subsets: (1) non-activated M0 macrophages; (2) classically-activated pro-inflammatory M1 macrophages; and (3) alternatively activated tissue-regenerative M2 macrophages [22]. In physiological bone healing, M1 macrophages take dominance in the early-stage osteogenesis, which can be gradually converted into M2 phenotype in later stage, and the timely M1-to-M2 phenotype switch during is considered an indispensable part of bone regeneration, which has been applied in developing bone regenerative approaches [22]. However, in aging condition, such a phenotypic switch is interrupted, and local macrophages exhibit a more inflammatory phenotype along with impaired bone regeneration, as compared with macrophages from bone healing site in the young [21,23–25]. This aging-associated inflammatory macrophages are detrimental for bone fracture healing [21], which contribute to the local inflammaging environment during aging bone healing. This phenomenon is due to the accumulation of senescent macrophages, which results in increased inflammatory secretome to induce the senescent phenotype in young cells; in addition, the declined phagocytosis in senescent macrophages hinders the physiological clearance of senescent cells, therefore further exacerbating the accumulation of senescence cells, forming a vicious circle to exaggerate inflammaging. This environment impedes bone regeneration in two mechanisms, on one hand, the chronical inflammatory condition is unfavorable for osteogenesis, on the other hand, the senescence of osteoblast progenitors further impairs the osteogenic differentiation. Therefore, macrophage is a major player in osteoimmunosenescence associated bone healing issues, which should be considered as a major target in developing bone healing biomaterials for the elderly. In addition, other immune cell types such as T cells are also involved in osteoimmunosenescence.

Currently how to improve aging bone healing remains a major gap in bone biomaterial design and development. Given the importance of inflammaging in bone healing difficulties in the elderly, it is therefore necessary to understand the mechanisms underlying the pathogenesis of inflammaging, and how inflammaging affects bone healing. This will guide the design and development of future materials to functionally improve aging bone regeneration by correcting inflammaging. Based on the perspectives listed above, the current review is drafted to summarize the recent findings regarding the detrimental roles of inflammaging in bone healing, the potential biomaterial-based approaches to resolve inflammaging, and to discuss the future directions in design and develop bone healing biomaterials for the elderly.

2. The Fundamental Role of Osteoimmunology in Bone Regeneration

It has long been realized that the immune and skeleton systems are intertwined together, which termed as osteoimmunology [26]. Bone is a dynamic tissue which undergoes a continuous remodeling including two coupled processes, known as osteoclast-driven bone resorption and osteoblast-driven bone formation [27]. Immune cells contribute to the maintenance of bone remodeling balance by modulating both bone resorption and formation, hence playing determinant roles in bone regeneration [26]. Cells from the adaptive system, especially the T-helper (Th) cells are known as key regulators in bone resorption. For example, Th cells under inflammatory stimulation are one of the major source of nuclear factor kappa B ligand (RANKL), an indispensable factor in osteoclastogenesis [28]. Th1 and Th17 cells contribute to osteoclastogenesis by producing pro-inflammatory cytokines such as IL-1, IL-6, IL-17, and TNF-α [29]. On the contrary, the regulatory T cells (Tregs) are found to inhibit osteoclastogenesis by secreting anti-inflammatory IL-10. The pro-inflammatory factors (such as IL-1, IL-6, IL-17, and TNF-α) have been reported to also induce osteogenesis, despite there are conflicting results, which indicates that the positive/negative effects on osteogenesis may be associated with the amount or duration of these factors in the microenvironment [29].

As one of the major players in the innate system, macrophage actively participate in bone remodeling modulation. Macrophages are key cells in driving the non-specific innate immune response and form a heterogenous population that possesses tissue-specific roles [30]. Macrophages may act directly, by destroying invading bacteria, parasites, viruses, and tumor cells, or indirectly, by releasing cytokines such as IL-1 and TNF-α, which can regulate other cells [31]. In addition, macrophage can clear the cells undergoing damage/apoptosis, a phagocytosis process via the scavenger receptors to recognize the oxidized proteins/lipoproteins [32,33].

Macrophages are also responsible for processing antigens and presenting digested peptides to T lymphocytes [34], and for tissue damage repair [35].

Macrophage is a type of plastic cells which can polarize towards a spectrum of phenotypes under different stimulations. The two ends of this spectrum are termed as inflammatory M1 (classically activated by lipopolysaccharides/LPS and interferon gamma/IFN-γ) and anti-inflammatory M2 (alternatively activated by IL-4 and IL-13) phenotypes, respectively [22,36]. M1 macrophages contribute to inflammation by releasing proinflammatory cytokines (e.g., TNF-α, IL-1, 6, 12, 15, 18, and 23), reactive oxygen species (ROS) and nitrogen intermediates (e.g., iNOS), producing prostanoids and matrix-degrading enzymes, inducing phagocytosis and antigen presenting [37,38]. This consequently triggers the activation of adaptive immune cells and induce osteoclastogenesis, that M1 macrophage can improve the differentiation and polarization of Th17 cells while reduce those of Treg cells, resulting imbalanced ratio of Treg/Th17 cells [39,40], a process enhances both inflammation and osteoclastogenesis. On the other hand, M2 macrophage is considered to reduce inflammation and improve tissue regeneration by releasing anti-inflammatory IL-10 and tissue regenerative TGF-β [41], therefore facilitating tissue healing by improving cell growth and the reconstruction of extracellular matrix (ECM) [32,42] (Figure 1).

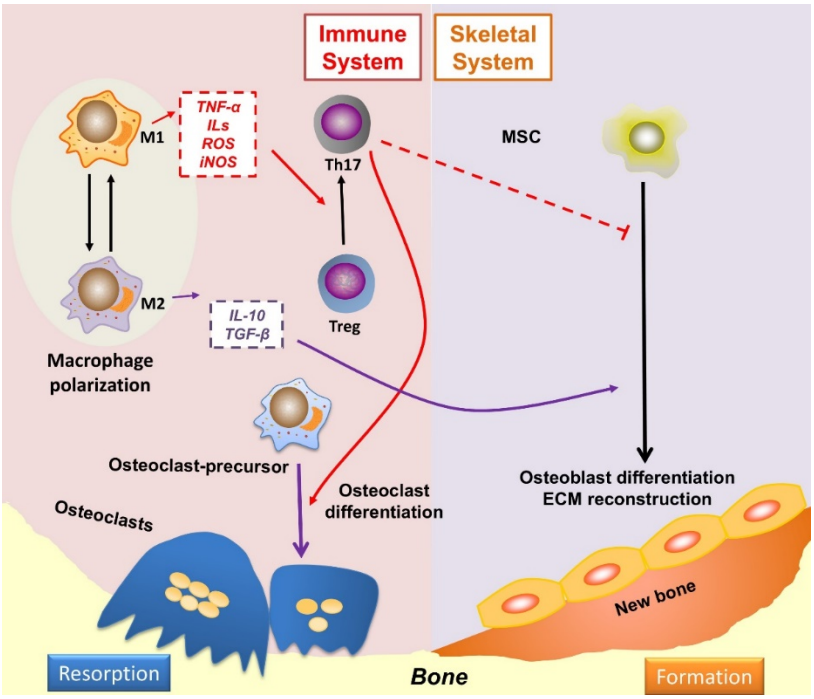

Figure 1. The central role of immune cells (macrophages) in regulating bone remodeling balance. The inflammatory M1 macrophage favors the polarization from Treg to Th17 cells, generating an environment favoring osteoclast differentiation over osteogenesis, thus enhancing bone resorption. On the other hand, the anti-inflammatory M2 macrophage favors osteoblast differentiation to facilitate bone formation. ILs: Interleukin-1, 6, 12, 15, 18, and 23.

The dynamic M1/M2 balance plays a decisive role in bone regeneration. In the early stage of natural bone healing, an evident M1-like macrophage infiltration and inflammation have been observed, which is considered as an essential part in bone healing [43]. Accordingly, conditioned medium (CM) from M1 macrophage enhanced osteogenesis as compared with the M2 macrophage-derived CM [44–50], suggesting M1 macrophage might trigger the initial osteogenic differentiation of mesenchymal stem cells (MSCs). Meanwhile, M2 phenotype becomes evident in the subsequent stage of bone healing, during which the early-stage inflammation is resolved gradually [43,51]. This M1-to-M2 transition is a vital part in bone regeneration [43,51], which has been utilized to in biomaterial design to develop osteoimmunomodulatory materials to induce an ideal immune microenvironment for bone regeneration [22]. The importance of timely M1-to-M2 transition is that it can facilitate a timely resolution of inflammation, as a microenvironment with a long-term inflammation may impede the generation and maturation of new bone tissue, which eventually becomes fibrous tissue. This could be evidenced from the fact that biomaterial resulting long-term inflammation showed poor osteoconductive effect [22]. Meanwhile, M2 macrophage has been recently found to play a decisive role in the subsequent bone maturing stage, by facilitating osteocyte maturation and determining the microstructure of newly-formed bone [52].

3. Inflammaging and Its Impact on Bone Healing

It has long been recognized that aging is associated a chronic, low-grade, sterile systemic inflammation that is referred to as "inflammaging" [53], as characterized by a 2-fold increase on serum levels of IL-1β, IL-6, TNF-α, and C Reactive protein (CRP) in the elderly (compared with the young individuals) [54–56], which is perceived to be a highly significant risk factor for both morbidity and mortality in the elderly [14]. Aging-associated systemic environmental changes such as accumulation of pathogen-related molecular patterns (PAMPs, due to aging-associated decline in tissue barrier functions) and damage-associated molecular patterns (DAMPs) [57,58] can be recognized by pattern recognize receptors (PRRs) in myeloid cells (especially macrophages) [59–61] to trigger their inflammatory responses (Figure 2A). Other changes such as higher levels of fatty acid and glucose in blood [62], hypercoagulation [63], can activate inflammatory response in immune cells, hence contributing to the pathogenesis of inflammageing. Furthermore, the senescent immune cells, especially senescent myeloid cells (neutrophils, macrophages, dendritic cells) play a central role in the pathogenesis of inflammaging [20,55] because of their intrinsic senescence to trigger their inflammatory response, due to the link between cellular senescence and inflammation mechanisms. In addition, the continuous accumulation of senescent cell population, due to paracrine senescence [18] to turn the young cells into their senescent phenotype, and the insufficient clearance of senescent cells by immune cells [64], further exacerbates the inflammaging condition in the aged people. Such a condition is detrimental to aging bone healing because a long-lasting inflammation favors osteoclastogenesis over osteogenesis (as explained in Section 1), thus creating an unfavorable environment to impede bone regeneration. For example, although M1-to-M2 transition can naturally happen in bone fracture healing of younger individuals, in the elderly, this transition could be significantly impaired by inflammaging, which consequently results in delayed union or non-union.

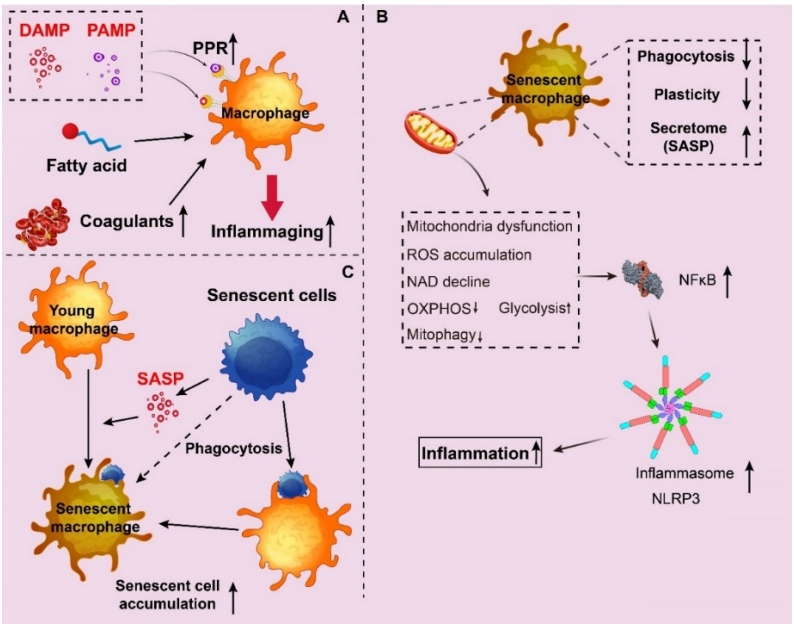

Figure 2. The central role of immune cells (macrophages) in the pathogenesis of inflammaging. Inflammaging can be associated to: (**A**) Inflammatory responses to aging associated systemic accumulation of DAMPs and PAMPs; (**B**) Intrinsic senescence of immune cells derived inflammatory secretome; and (**C**) Paracrine senescence to induce senescence in young cells, which, in combination with the declined phagocytosis (clearance) of senescent cells by senescent immune cells, can amplify the accumulation of senescent cells and inflammaging.

3.1. Immunosenescence

Cellular senescence (both immune and tissue cells) can be characterized by abnormal cell behaviors such as irreversible cell cycle arrest, loss of replication capacity, resistance to apoptosis; morphological change such as global cell enlargement, defective nuclei and organelles such as misshaped nuclei, chromosome reorganization, telomere shortening, protein aggregation in endoplasmic reticulum, enlarged and dysfunctional mitochondria, and nonfunctional lysosomes/proteasome system; and molecular changes such as DNA damage, over production of ROS, inflammatory secretome (SASP), energy metabolism alterations (e.g., shift from oxidative phosphorylation (OXPHOS) to glycolysis [12,65]), decline in oxidized nicotinamide adenine dinucleotide (NAD^+) level, reduced autophagy, etc. [66–69]. Currently the molecular signaling pathways underlying how these events lead to

inflammation in senescent cells remain to be further explored. They can activate NF-κB signaling pathway and NLRP3 inflammasome (both are fundamental upstream signals of SASP production) [70,71], thereby consequently triggering the production of SASP to recruit immune cells and induce their inflammatory response to contribute to inflammaging (Figure 2B). Especially, ROS are recognized hallmark of cellular senescence [69]. ROS are generated by the partial reduction of oxygen, are a group of radical/non-radical oxygen species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•) [72]. ROS are overproduced in senescent cells. Meanwhile, the levels of ROS clearing enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), are decreased [73], which further facilitating ROS accumulation to cause oxidative damage to macromolecules such as DNA, lipid, and protein, thereby exacerbating cellular senescence. ROS accumulation can significantly promotes chronic inflammatory response and SASP secretion [12,74–77] through the two major pathways of Toll-like-receptors (TLRs), and NLRP3 inflammasome [77,78]. Thus, ROS are considered as the major reason for inflammaging.

Human survival is inextricably linked to a functional immune system, which protects the host against infections and malignancies, whereas the immune system deteriorates along with aging, mainly due to the aging associated senescence in immune cells, and these remarkable changes collectively are known as immunosenescence [78]. Many immune cell types senesce with aging (immunosenescence), featured by increased expression levels of $p16^{INK4a}$ and $p21^{CIP1}$, inflammatory secretome (SASP), and dysregulated immune responses [64,79,80]. Immunosenescence can lead to a poor response to pathogenic infections and malignancies, and a diminished response to vaccination [69]. The innate immune system is the body's first line of defense, providing fast and effective immune responses against invading pathogens in a non-specific mechanism [81]. The adaptive immune system functions efficiently to eliminate pathogens that escape the innate immune system by precise recognition of antigen, memory formation, and antigen-specific immunity [82]. Immunosenescence affects both innate and adaptive immune systems, leading to increase susceptibility to viral and bacterial infections and contributing to the development of age-related diseases, such as cancer, metabolic syndrome, atherosclerosis, and neurodegeneration [83].

The impact of immunosenescence on the adapt immune system is generally recognized as a decline in immune function [55], as characterized by changes in the naive: memory T cell ratio, CD4:CD8 ratio, impaired calcium-mediated signaling and thymic atrophy [84]. On the other hand, the senescent innate immune system exhibits an induced inflammatory-like response to act as the major player in inflammaging [55]. In addition, the senescence of hematopoietic stem cell results in a skewed differentiation potential from lymphoid linage to myeloid linage [55,85–87], resulting in increased myeloid cell numbers while decreased lymphocyte population in the elderly (as compared to young individuals). This, in combination of the functional decline in the adaptive system, make the innate system as the main inflammatory mediator in response to stimulations [55], among which macrophage (and its precursor monocyte) population are considered as the central player in initialing inflammaging [20,55].

Given the importance of macrophages to orchestrate immune responses and tissue homeostasis, macrophage function is dysregulated with aging, a process known as MacrophAging, contributes substantially to the dysfunctional immune responses observed in the elderly [88]. Senescent macrophages are characterized as inflammatory-like phenotype with NLRP3 inflammasome activation, defective capacities of phagocytosis and antigen presentation, mitochondrial dysfunction, and impaired cellular metabolism [65,70,89–91]. Renshaw et al. has shown that splenic and activated peritoneal macrophage from aged mice secreted substantially lower amount of IL-6 and TNF-alpha when stimulated with a range of TLR ligands compared to macrophages from young mice, indicating impaired phagocytic capacity of macrophages upon age [92]. Senescent macrophages exhibit a variety of age-associated immune dysfunction, including a decrease in oxidative and nitric oxide (NO) burst and reduced endocytic and phagocytic capacities [93]. A recent study has demonstrated that the macrophages derived from bone marrow in aged rats are hyper-responsive to two potent inflammatory stimuli LPS and IFN-γ [94]. Similarly, when exposed to the inflammatory microenvironment that develops in brain with age, macrophages/microglia are likely to propagate the existing inflammation and contribute to a damaging cascade that negatively impacts neuronal function [94].

3.2. The Interaction of Immune and Senescent Tissue Cells Exacerbates Inflammaging

Senescent tissue cells exist in both young and aged individuals and can be efficiently cleared by immune cells such as phagocytes (e.g., macrophages) in the young. In the aging population, however, the senescent immune cells are unable to clear the senescent tissue cells due to a lack of phagocytosis capacity [64], which consequently results in accumulating senescent cell population in the local tissue. This contributes to a pathogenesis of local

senescent environment to trigger inflammaging by two main mechanisms, one is the intrinsic senescence of immune cells (such as macrophages) to trigger the inflammatory response, the other is the interplay between immune cells and senescent tissue cells via SASP. Senescent tissue cells secrete SASP to recruit and activate the inflammatory response of young immune cells to further enhance inflammaging. More importantly, senescent cell-derived SASP can induce senescence in the neighboring young cells [95], a mechanism termed as paracrine senescence [18] to exacerbating both senescent tissue cell accumulation and inflammaging (Figure 2C).

Senescent macrophages are critical inducers of inflammaging which exhibit long-lasting inflammatory phenotypes [88]. Moreover, macrophage is considered as a major immune component to interact with senescent tissue cells. As one of the main executors in phagocytosis, macrophage population play a major role in clearance of damaged cell debris/components and senescent tissue cells. In young individuals, that senescent tissue cells can be efficiently cleared by macrophages to avoid a long-term inflammatory microenvironment, and to prevent the young tissue cells from paracrine senescence. However, in aging individuals, the senescent macrophages are dysfunctional and unable to clear senescent tissue cells due to a lack of phagocytosis capacity [64]; meanwhile, the phagocytosis of senescent tissue cells can induce the senescent phenotype in young macrophages [96], which therefore resulting in accumulation of senescent tissue cells, senescent macrophages and SASP, forming a feed-forward cycle to amplify both inflammation and paracrine senescence.

3.3. The Influence of Imflammaging Environment on Bone Regeneration

In the aging bone environment in vivo, senescent immune cells have been identified in populations such as T cell, B cell, and cells from the myeloid lineage, however, the myeloid cells (including macrophages, neutrophils, and granulocytes) were found as the main immune contributor to the high level of SASP in aging bone [97]. Kim and colleagues found a significant increased number of pro-inflammatory macrophages in bone marrow of aged mice compared with that in young mice and observed age-related dysregulation of pro- and anti-inflammatory cytokines in bone marrow-derived macrophages [98]. Indeed, a recent study has compared the macrophage phenotype in fracture healing models of aging and young mice, and found that macrophage infiltration was induced in fracture callus of aging mice. The fracture callus macrophages from old mice are transcriptionally distinct from those from young mice, with an up-regulation of M1/pro-inflammatory genes and impaired phagocytosis in macrophages from old mice [21]. These M1-like macrophages was found to result in delayed fracture healing in aging mice, as demonstrated by the phenomenon that their depletion significantly improved aging bone healing [21]. This study suggests the senescent macrophage should be a major contributor to the impaired bone healing in the elderly.

Therefore, in aging bone a pathological microenvironment consists of long-term chronical inflammation is generated, with senescent macrophages considered as the major source of inflammaging [20,55], which is unfavorable environment for bone regeneration. In aging patient, the interplay between senescent immune and skeletal cells, namely osteoimmunosenescence, is expected contributes to a prolonged/chronical inflammatory environment after bone injury (e.g., fracture) to impede osteogenesis (Figure 3A,B). As explained in Section 2, such a condition is detrimental for bone regeneration, which favoring bone resorption over bone formation. Accordingly, the capacity of bone regeneration in response to injury declines with advancing age. According to clinical observation, impaired bone defect healing and increased rates of nonunion are often seen in elderly patients due to protracted chronic inflammation [99]. Specifically, an impaired expression of anti-inflammatory M2 macrophage markers in bone defect hematoma from aged rats is associated with poor revascularization in the early callus, leading to compromised bone healing [23]. This age-associated inflammation is believed to diminish the regenerative potential of skeletal stem/progenitor cells (SSPC), suggesting that modification of the inflammatory microenvironment could be a translational approach to reinvigorate the aged stem cell functions for improved bone healing outcomes in the elderly patients [100]. For example, by resolving inflammaging via inducing an anti-inflammatory macrophage phenotype, the tibial fracture healing was significantly improved in aging mice [101].

Although adaptive immune cells are not considered as the main source of SASP in aging bone [97], a recent study found the local adaptive immune response during fracture healing differs with age [102] (using a mice fracture healing model). Compared with young mice (12-week), middle-aged mice (52-week) showed a reduced facture healing while induced infiltration of Th cells and cytotoxic T cells in fracture callus, although no difference on Treg cells. More importantly, both systemic and local application of immunomodulator to inhibit inflammation, the fracture healing in middle-aged mice was significantly accelerated [102], suggesting the local adaptive immune cells might impede bone healing via inducing an inflammatory-like environment in the middle-aged mice. Although this study did not use an aging animal model, it suggests that the local adaptive immune system in bone

changes along with age-growth, and such change may be associated with inflammaging and the impaired bone healing in the elderly, which needs further investigation in the future.

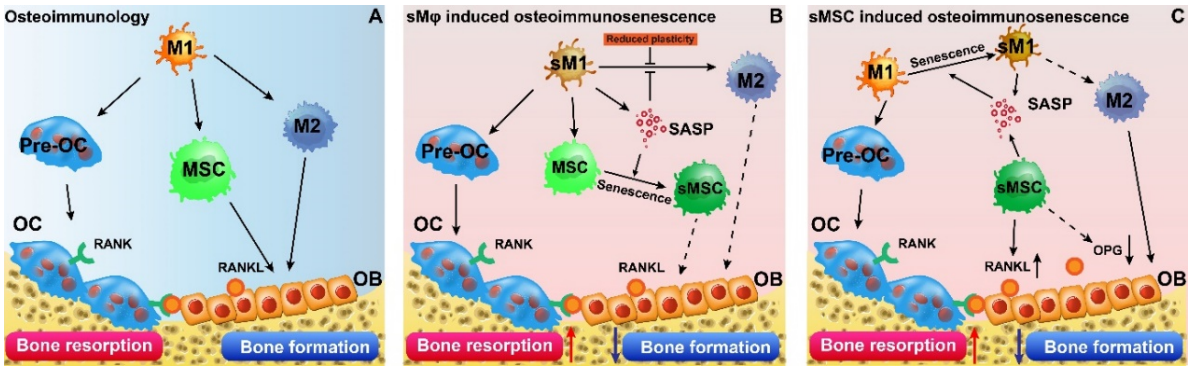

Figure 3. Schematic illustration of the osteoimmunology in normal and senescent conditions (osteoimmunosenescence). (**A**) In normal conditions, the crosstalk between immune (e.g., macrophage/MΦ) and skeletal (e.g., MSC) cells contribute to a balanced bone remodeling. However, in senescent conditions, (**B**) senescent macrophages (sMΦ) can stay in the inflammatory M1 phenotype (sM1), due to the released SASP and reduced plasticity to inhibit the M1-to-M2 phenotype switch. This can result in an immune environment that favors osteoclastogenesis over osteogenesis. Meanwhile (**C**), SASP can turn MSCs into senescent cells (sMSCs) with impaired osteogenic capacity, induced RANKL production and reduced OPG expression, therefore further contributing to the imbalance between bone formation and formation. OB: osteoblast; OC: osteoclast.

3.4. Senescent MSCs Resulted from Osteoimmunosenescence

In aging bone environment, despite the interplay between immune and skeletal cells to determine bone remodeling, osteoimmunosenescence can induce senescence in the young skeletal cells via paracrine senescence [95]. The paracrine senescence exists in senescent and young skeletal cells, for example, senescent (Passage: 10) MSCs derived conditioned medium induced senescence in young MSCs (Passage: 1) [103,104]. The accumulation of senescent skeletal cells can in-turn forming a positive feed-back to amplify the inflammaging environment, by activating and stimulating the immune cells, and inducing their senescence.

Despite for macrophages, senescent skeletal cells are critical sources of SASP in bone. Senescence has been found in cells from the skeletal system of aging mice, such as osteoblast progenitors (MSCs), osteoblasts and osteocytes [97]. Compared with the young (< 16 years old), MSCs harvested from older individuals (>40 years old) showed significant induced secretion of inflammatory cytokines, which were further characterized as 27 SASP components [104,105]. The secretion of SASP is considered to impede the inflammation-suppressive capacity of MSC in aging individuals [104,105], which may further contribute to the inflammatory environment in aging bone marrow. Joshua et al. found that among the mesenchymal lineage cells in aging bone environment, osteocyte population showed more severe senescent phenotype than MSCs and osteoblasts (as indicated by the expression of $p16^{Ink4a}$), more importantly, osteocytes have been identified as the major source of SASP in aging mice bone microenvironment (other major SASP sources are myeloid cells) [97]. The reason for this phenomenon could be that, compared with MSCs and osteoblasts, osteocytes have much longer lifespan and therefore tend to be affected more by aging [106]. As a cell embedded in mineralized matrix, osteocyte highly depends on autophagy to maintain its physiological status [29], whereas the impeded autophagy in senescent cells [107] might affect aging osteocyte physiology. Interestingly, the expression of autophagy markers Atg7 and LC3 were significantly reduced in osteocytes and myeloid cells in aging mice (as compared with osteocytes and myeloid cells from young mice) [97], suggesting a strong relationship between inflammaging and autophagy-deficiency.

Except for exacerbating inflammaging (to generate an unfavorable healing environment), the senescence of skeletal cells can directly impede bone regeneration by dysregulating bone remodeling [104]. The senescent MSCs, as characterized with reduced cell proliferation and osteogenic differentiation compared with young BMSCs [104,108], contribute to the impaired bone healing in aging population. Interestingly, a major reason for the retarded osteogenic differentiation in senescent MSCs is that they favor adipogenesis over osteogenesis, which is in accordance with the phenomenon that adipose tissue increases along with age growth [104,108]. Cellular senescence associated high levels of ROS result in oxidative stress which can impair several cellular and molecular mechanisms in bone healing. Exposure to high levels of ROS would reduce the self-renewal capacity of MSCs, promote the activation of PPARγ to induce MSC differentiation toward adipose cells and, inhibit β-catenin signaling pathway-dependent osteoblast differentiation. MSCs affected by extreme oxidative stress have shown

the failure of osteogenesis and resulted in impaired bone formation and decreased bone mass [109–111]. In addition, the accumulation of senescent MSCs with inferior osteogenesis capacity [104] can further dampen bone healing. In addition, along with age growth (from 6 weeks to 24 months), MSCs and osteoblastic cells from mice bone marrow were found to facilitate bone resorption by increasing the production of osteoclastogenic RANKL while decreasing that of OPG (which is anti-osteoclastogenesis) [112], which may impair bone regeneration by breaking the bone remodeling balance (Figure 3C).

As indicated above, a better understanding of osteoimmunosenescence is necessary to mitigate bone loss and improve bone healing with aging. Thus, it is not unreasonable to expect that this information will continue to be translated into therapies to rejuvenate/modulate the aging immune system for better bone repair and regeneration. The osteoimmunosenescence is therefore considered as a major obstacle in aging bone healing, which not only contributes to an unfavorable inflammatory condition for osteogenesis, but also develops a "senescent domain" to turn the young immune and mesenchymal cells into senescence, forming a vicious cycle to magnify inflammaging and to further deteriorate the bone environment. Thus, to facilitate aging bone healing, two key points should be considered to target osteoimmunosenescence in bone biomaterial design, one is to modulate the unfavorable immune environment, the other is to target the cellular senescence.

4. Current Advances in Biomaterials Development Targeting Immunomodulation

Owing to the detrimental role of inflammaging in bone healing, immunomodulation (to target macrophage polarization) is therefore considered as a necessary and effective approach to improve bone regeneration in the elderly. The osteoimmunomodulatory biomaterials (to regulate macrophage polarization), which were proposed in our previous work [22], have been extensively developed and investigated in recent years. These materials can be categorized as nanomaterials and implant materials. Nanomaterials mainly regulate the M1-to-M2 polarization by delivering immunomodulatory biomolecules; meanwhile, their physiochemical properties (e.g., composition, size, structure, surface, and protein aggregation) can affect the macrophage polarization. Similarly, the physiochemical properties of implants materials, including surface property, structure, modification, material porosity, and released ions, can impact macrophage polarization and local immune environment. This chapter will summarize the current advances in osteoimmunomodulatory biomaterials, which can be considered in biomaterial design for the aged patients.

4.1. Nanomaterials

Nanotechnology has emerged as a novel alternative to stir up the field of bone tissue engineering, which facilitates the development of nanoengineered materials in form of particles, fibers, tubes, spheres, composites, porous materials and structured coatings [113,114]. Taking nanomaterials as a basis, nanotechnology can create both drug or molecule delivery systems or even facilitate tissue regeneration by mimicking native milieu [115–117]. Firstly, nanomaterials have shown great potential as delivery platforms, enabling drugs to reach the target site avoiding systemic side effects and reducing administered doses [117,118]. Secondly, it is known that micro- and nano-environmental signals from biomaterials can alter cell-biomaterial interaction [114], so resembling the natural architecture of bone using nanomaterials can be a key choice to improve tissue regeneration [116]. The immunomodulatory nanomaterials can modulate the immune cell response (the M1-to-M2 macrophage polarization) to create a favorable local environment for osteogenesis. Nanomaterials for immunomodulation to regulate the M1-to-M2 macrophage polarization have been investigated for bone tissue engineering [114], and their physiochemical properties (composition, size, structure, surface, and aggregation) can affect the macrophage polarization. On this wise nanomaterials could be an appropriate tool to obtain a great biomaterial-mediated immunomodulation approaches for bone regeneration. During these lines the latest advances on nanomaterials for immunomodulation will be described and discussed.

4.1.1. Nanomaterial-Based Drug Delivery of Immunomodulatory Factors

A review of recent studies makes it clear that nanomaterials have been widely used as delivery-platforms in different areas of biomedicine. Along the previous lines, the latest advances in nanomaterials for immunomodulation have been seen for bone tissue engineering [114]. Most studies use nanomaterials (e.g., liposomal nanoparticles or mesoporous silica nanoparticles) to delivery different types of cargos, to achieve a sustainable and local release of drugs, ions, GFs, genes, etc., for immunomodulation (examples listed in Table 1). Nonetheless, the vast variety of nanomaterials and cargos, as well as the variability between different studies, makes it difficult to create a final conclusion, so it would be necessary to do more in-depth studies of each delivery-platform so as to ensure biosecurity and efficacy for future use in tissue engineering; meanwhile, challenges such

as scalability, reproducibility, or how to optimize the release profiles of therapeutic cargos should be considered in future design of immunomodulatory nanomaterials.

Nanomaterials to Deliver Immunomodulatory Biomolecules

In the past decades, the advances in nanotechnology have developed nanomaterials with suitable properties to ensure a controlled delivery of myriad molecules including drugs, cytokines and growth factors [117], which not only prevents the molecules from degradation, but also allowing for a sustained release of the molecules to achieve a long-term therapeutic effect and meanwhile, to avoid side-effects caused by burst-release. This is critical for bone regeneration, as explained in Section 2 and our previous work [36], a sudden shutdown of early-stage inflammation, which can be induced by burst-release of immunomodulators, is not ideal for bone regeneration, since such an environment is vital for initializing both osteogenesis and angiogenesis [43]. Instead, a sustained release of immunomodulators can restrain the inflammation at certain level to ensure a timely inflammation resolution at later-stage of bone regeneration [36]. These nanomaterials have been used as carriers for the delivery of anti-inflammatory drugs and bioactive molecules implied in the crosstalk between skeletal and immune cells [113].

Owing to the importance of M1-to-M2 macrophage phenotype switch in bone regeneration, nanomaterials have been designed to deliver IL-4, a recognized cytokine to induce M2 polarization. For instance, Jin et al. developed a hierarchical intrafibrillarly mineralized collagen scaffold with a bone-like staggered nanointerface, which could be used to load IL-4 (due to its porous structure to ensure a good capacity of water absorption) to significantly promote bone regeneration in vivo, via a functional immunomodulation by inducing $CD68^{+}CD163^{+}$ M2-like macrophage polarization [119]. Our previous work [120] developed titanium dioxide (TiO_2) nanotubes for the controlled co-delivery of a osteogenic RGD peptide and anti-inflammatory IL-4. The findings revealed that the functionalized nanotubes simultaneously enhanced MSCs recruitment/osteogenic differentiation and switched macrophages phenotype to anti-inflammatory M2, which allowed a synergistic osteoimmune microenvironment enhancing early osteogenesis. In addition to IL-4, the delivery of osteogenic BMP-2 has been found to exert an osteoimmunomodulatory effect. For example, Vantucci et al. [121] proposed heparin methacrylamide nanoparticles (HMPs) to be used for the spatiotemporal delivery of BMP-2. The strong binding capacity between HMPs and BMP-2 allowed to deliver the osteogenic growth factor in controlled doses and stimulate immune T cells to secrete cytokines actively involved in bone cells activity and tissue regeneration.

Besides engineered nanosurface/nanostructure, micro/nanoparticle-based drug delivery system has been extensively investigated. In a recent study, a zeolitic imidazolate frameworks (IL@ZIF) nano-platform was developed to deliver the anti-inflammatory IL-33. The nano-platform-derived Zn^{2+} can reduce the ROS level in macrophages to alleviate inflammation-associated oxidative stress; moreover, Zn^{2+} activates the IL-33 receptor on macrophage to generate a synergic effect to facilitate IL-33 directed immunomodulation to promote tissue regeneration in diabetic mice (a special condition with chronical inflammation) [122]. In another study, a self-assembled nanofibrous heparin-modified gelatin microsphere (NHG-MS) was developed to load IL-4, due to the heparin binding domains in IL-4 to facilitate the loading and stabilization of IL-4, thereby protecting IL-4 from degradation to ensure a sustained release to modulate macrophage polarization, generating an osteoimmunomodulatory effect to improve bone regeneration under diabetic mellitus conditions [123].

The combination of scaffold/implant materials with nanoparticles have been proposed to stimulate osteogenic and immune cells co-working [124,125]. For example, Li et al., developed three-dimensionally (3D) printed liposomes loaded with aspirin (Asp@Lipo) and deposited them in a Polycaprolactone (PCL) scaffold (PCL-Asp@Lipo) [126]. Authors demonstrated its ability to regenerate bone with different in vitro and in vivo studies, the latter ones manifested that the PCL-Asp@Lipo promoted immunomodulation due to decreased TNF-α and IFN-γ concentrations in a subcutaneous rat model [127]. He et al. developed peptide IL-37-loaded silk fibroin nanoparticles (SFNPs) immobilized on the surface of a titanium (Ti)-based material and showed that the SFNPs-modified Ti samples improved the paracrine signalling between MSCs and macrophages for a superior anti-inflammatory response and improved bone formation in vivo [125]. Bai et al. showed that microporous Ti surfaces coated with nano-hydroxyapatite (HA) positively modulated inflammation and improved the osteoimmune microenvironment for angiogenesis and osteogenesis in bone remodelling [124].

Except for synthesized nanoparticles, cell-derived exosomes (EXO), a type of natural nanostructures, have been used as vehicles to deliver growth factors and anti-inflammatory cytokines. For example, EXO have been used to load TGF-β1 and IL-10 to inhibit degenerative bone disease [128]. Authors isolated exosomes from dendritic cells, embedded them with the two immunoregulatory molecules mentioned above, and administered them both intravenously and locally to reprogram the Th17 cell mediated immune response with the aim of attenuating alveolar bone loss. Findings revealed that EXO protected these molecules from degradation, thus those

molecules could perform their synergistic regulatory effect, and a reduction on osteoclast-derived bone degeneration was then achieved [128].

Nanomaterials could also be used for gene-delivery. Especially for metabolic skeletal disorders such as osteoporosis, nanomaterials have shown potential to enable gene-therapy. For example, Li and associates fabricated Poly (anti-inflammatory salicylic acid) nanoparticles (PSA-NPs) to deliver microRNA-21 (miR-21) (a microRNA beneficial for osteogenesis) [129]. To own miR-21 targeting ability, nanoparticles were treated with $(Asp\text{-}Ser\text{-}Ser)_6$ $(DSS)_6$ peptide. Once the system was constructed, authors proved its ability to improve bone regeneration in a mice osteoporotic bone model. Findings demonstrated that miR-21@PSA-NP-$(DSS)_6$ significantly reduced TNF-α and IL-6 –both pro-inflammatory cytokines- levels, reducing the inflammatory environment created in osteoporosis. Aside from that, the delivery of miR-21 enhanced osteogenesis too, so authors concluded that miR-21@PSA-NP-$(DSS)_6$ system showed great potential to improve osteoporosis enhancing both anti-inflammatory effect and pro-osteogenic effect [129].

Nanomaterial for Environmental-Responsive Drug Release

In recent years, the concept of smart material has facilitated the design of second-generation nanomaterials with more precise site-specific controlled release [130]. For example, the strategies for controlled drug release induced by near-infrared laser irradiation have been extensively investigated, which allow for a switchable drug release at certain time point. The NIR-responsive nanomaterials include gold nanoparticles (e.g., gold nanocage, gold nanorod, gold nanoshell, etc.) and carbon dots (CDs) [131], which have been used in tissue engineering application [132]. Especially, the development of environmental-responsive drug delivery system has realized a precise immunomodulation, which can release different amounts of immune regulators in response to different inflammation levels, therefore allowing for a potential personalized osteoimmunomodulation. This is critical for the translation of these material, since due to the different physiological/pathological conditions in each individual, a traditional drug delivery system is difficult to achieve an ideal osteoimmunomodulation for each patient to maximize bone regeneration.

Recently, the features of inflammatory environment such as low pH (pH range: 5.0–6.0) [133] and high ROS levels have been utilized to develop inflammation-responsive nano-systems. For example, a pH and ROS dual-responsive drug delivery nano-system was developed to smartly release on-demand drug in response to inflammation [134]. This nano-system is developed by grafting 3-carboxy-phenylboronic acid to the gelatin molecular backbone (via amide bond formation between −COOH from 3-carboxy-phenylboronic acid and −NH2 in gelatin gel and via formation of an amide bond between −NH2 in Gel and in 3-carboxy-BA), cross-linking with poly(vinyl alcohol)(containing rich *o*-diols), and then encapsulation with vancomycin-conjugated silver nanoclusters (VAN-AgNCs) and pH-sensitive micelles loaded with nimesulide (NIM). The rapidly formed dynamic phenylboronic acid–diol ester bonds endow the gel with pH- and ROS-responsive inflammation control. This nano-system successfully promoting tissue regeneration in diabetic mice via smart inflammation and infection control [36,134]. Similarly, a pH-responsive self-assembled iron-catechin nanoparticles (Fe-cat NPs) were developed based on the coordinated reaction between iron ions and catechin, which can disassemble and release catechin intracellularly in response to low pH environment in lysosome [135]. The released catechin can not only enhance osteogenesis of stem cells, but also induce the M1-to-M2 macrophage phenotype switch to generate an ideal osteoimmune microenvironment. Considering the lower pH of inflammatory microenvironment [133], it is then expected that this nanomaterial can be used as inflammation-responsive controlled-release system for advanced osteoimmunomodulation. Future investigations should focus on developing specific inflammation-responsive nano-systems, such as enzyme-responsive [130] and redox-responsive [130] materials, to enhance osteoimmunomodulation.

Table 1. Nanomaterials to deliver immunomodulatory biomolecules.

Nanomaterials	Regulatory Effects	Loaded Immunomodulatory Biomolecules	Application	Ref.
Collagen scaffold with bone-like staggered nanointerface	M1-to-M2 macrophages phenotype switch	IL-4 (porous structure for solution absorption)	Bone regeneration	[119]
TiO_2 nanotubes	M1-to-M2 macrophages phenotype switch MSCs early osteogenic differentiation	Co-delivery of RGD peptide and IL-4 (tube structure)	Bone regeneration	[120]

Table 1. *Cont.*

Nanomaterials	Regulatory Effects	Loaded Immunomodulatory Biomolecules	Application	Ref.
Heparin methacrylamide nanoparticles	Immune T cells stimulation for cytokines delivery	BMP-2 deliver in controlled doses (via heparin binding)	Bone regeneration	[121]
Nanofibrous heparin-modified gelatin microsphere	Anti-inflammatory macrophage polarization	IL-4 (via heparin binding)	Bone regeneration	[123]
Zeolitic imidazolate frameworks nano-platform	Anti-ROS and anti-inflammatory macrophage response	Synergic effect from co-delivery of Zn^{2+} and IL-33	Diabetic tissue regeneration	[122]
Polycaprolactone scaffold deposited with aspirin-loading liposome	Inhibition on inflammatory cytokine release	Aspirin (entrapment)	Bone regeneration	[126]
IL-37-loaded SF nanoparticles-modified Ti samples	Superior anti-inflammatory response Enhanced paracrine signalling between MSCs and macrophages	IL-37 (entrapment)	Bone regeneration	[125]
Dendritic cell-derived exosomes	Inflammation suppression by regulating Th17 cells	TGF-β1 and IL-10 (entrapment)	Reduction on bone degeneration	[128]
miR-21@PSA-NP-$(DSS)_6$	Anti-inflammatory and pro-osteogenic effects	miR-21 (via $(Asp\text{-}Ser\text{-}Ser)_6$ $(DSS)_6$ peptide)	Bone regeneration under osteoporosis condition	[129]
Hydrogel@VAN-AgNCs&MIC	Anti-inflammatory macrophage response	Nimesulide (released in response to low pH and high ROS (inflammatory) environment)	Diabetic tissue regeneration	[134]
pH-responsive self-assembled iron-catechin nanoparticles	M1-to-M2 macrophages phenotype switch Osteogenesis of stem cells	Catechin (released intracellularly in response to low pH environment in lysosome)	Bone regeneration	[135]

TiO_2: Titanium dioxide; IL-4: Interleukin-4; IL-10: Interleukin-10; IL-33: Interleukin-10; IL-37: Interleukin-37; BMP-2: Bone morphogenetic protein 2; MSCs: Mesenchymal stem cells; TGF-β: Transforming growth factor-β; SF: Silk fibroin; miR-21@PSA-NP-$(DSS)_6$: Poly (anti-inflammatory salicylic acid) nanoparticles (PSA-NPs)-$(Asp\text{-}Ser\text{-}Ser)_6(DSS)_6$ peptide for delivery of microRNA-21 (miR-21); Hydrogel@VAN-AgNCs&MIC: A pH- and ROS-dual responsive hydrogel loaded with vancomycin (VAN)-loading Ag nanoclusters (AgNCs) and nimesulide (NIM)-loaded micelle.

Cell Membrane-Coated Nanomaterial

Inspired by natural biology, cell membrane-mimetic surface engineering and cell membrane camouflaging technology have been widely investigated to develop the third-generation nanomaterials known as the cell membrane camouflaged nanomaterials. These nanomaterials are produced by transferring the biological features of cells to synthetic the materials formulations, which has gained unprecedented attention in biomedical fields. Compared with the first- and second-generation nanomaterials, these biomimetic nanomaterials are bestowed with favoured properties of elongated circulation time, immune evading and active targeting, which exhibit great potential in numerous biomedical applications and are anticipated to revolutionize the traditional nanomedicine [136]. They can be developed through novel extraction processes focused on the direct use of the membrane proteins of native cell types to be incorporated into the surface of nanomaterials [137]. As a result, the surface features of cells are expected to be expressed at the nanomaterial surface, mediating the interactions with circulating immune cells and primary cells of specific tissues.

In a previous study [138], the cell membrane of BMSCs was used to cover the surface of Fe_3O_4 nanoparticles encapsulating Kartogenin (KGN), a drug commonly used for cartilage repair and regeneration. These biomimetic stem cell nanovehicles showed excellent biocompatibility and when injected at the intra-articular knee of a cartilage defect rat model showed higher regeneration capabilities as compared to pure KGN due to the internal natural activities of BMSCs membrane. Moreover, a controlled immune response was achieved, as the local macrophages showed no obvious inflammatory response owing to the potential immunomodulatory effect of BMSC membrane, indicating the positive cell communications triggered by the stem cell membrane-coated

nanovehicles. Building on the idea of membrane-coated nanoparticles, Zhang et al., [139] developed neutrophil membrane-coated nanoparticles applied as decoys of neutrophil-targeted biological molecules capable of neutralizing pro-inflammatory cytokines involved in the activation, migration, and recruitment of neutrophils to the joints, which suppressing synovial inflammation and joint damage in in vivo arthritis models.

Moreover, cell membrane camouflaged nanomaterials have been used in drug delivery. For example, the LPS pre-treated macrophage cell membrane was used to coat nanomaterials to compete with macrophages to bind with inflammatory cytokines TNF-α and IL-6. The biomimetic anti-inflammatory nano-capsule (BANC) was developed by using LPS pre-treated macrophage cell membrane to engulf gold nanocage, a system to deliver resolvin D1 (RvD1, a drug for inducing M2 macrophage polarization). The in vitro and in vivo release of findings revealed that BANC was able to transport and release RvD1 factor in a precise way through under NIR irradiation, which enabled an enhanced M2 macrophage polarization to benefit bone regeneration. More importantly, the coating membrane which containing TNF-α and IL-6 receptors can compete with immune cells to bind with the two inflammatory cytokines, thereby alleviating the overall inflammatory response to benefit bone regeneration [132]. Similarly, inspired by the inflammation-targeting capacity of macrophages, another study [140] developed cell membrane-mimetic surface nanoparticles for the targeted delivery of therapeutics applied in rheumatoid arthritis (RA). The macrophage-derived microvesicles were used for coating poly(lactic-co-glycolic acid) (PLGA) nanoparticles, which exhibiting a similar bioactivity to that observed on RA-targeting macrophages. Moreover, the macrophage membrane-coated PLGA nanoparticles were capable to bind to inflamed human umbilical vein endothelial cells (HUVECs) and targeting arthritic tissue in a mouse model.

Taken together, the cell membrane-coating is a biomimetic innovative technology which can be utilized to improve the functionality of traditional nanomaterials (especially nanoparticles) to become more biocompatible, efficient for drug delivery and immune modulation. This technology should be further explored in future studies to provide an expansive option for tissue regenerative applications including cartilage, bone and osteochondral tissues.

4.1.2. Effects of Nanomaterial Features on Host Immune Response

The interactions occurring between nanomaterials and the immune system are critical to the development of these special biomaterials, in such a way that their efficacy and safety can be altered by nanomaterial features [126,141,142]. After entering the body, nanomaterials are immediately recognized by the innate immune system by interacting with several biological components, such as cells, receptors or proteins, causing the activation of cell signalling cascades, and subsequently resulting in unexpected immune responses and even harmful outcomes (e.g., autoimmune diseases or cancer) [143]. The immune system recognizes nanomaterials by their composition and surface properties. In fact, several physicochemical properties, such as composition, size, shape, surface topography, aggregation and charge, play a role in the way how the immune system responds to nanomaterials (e.g., activation, suppression or clearance by the immune cells)(Figure 4) [144]. For example, it has been show that the shape and surface chemistry of gold nanoparticles (AuNPs) strongly influenced the uptake and the expression of inflammation-related genes, by the interface affinity between cells and nanorods/nanospheres [145]. In what concerns surface hydrophobicity, hydrophilic nanomaterials seem to alleviate inflammatory immune reactions as compared to lipophilic nanomaterials [146]. Therefore, materials properties can be modulated to adjust the interactions occurring between the nanomaterials and immune system [117,147].

One of the consensuses regarding the use of biomaterials for osteoimmunomodulation is that new tissue engineering applications should possess immunomodulatory functions, i.e., have the ability to remodel the immune environment and recreate the tissue regeneration process [22]. However, a major limitation of the modulation of nanomaterial properties is that the parameters affecting the immunological properties of nanomaterials are interrelated [144]. For example, protein adsorption is interconnected with the surface charge of nanomaterials (e.g., it decreases as the surface charge increases), and inserting different functional groups to the surface of nanomaterials to modify their surface charge may also affect their hydrophobicity. Within this section, we reviewed the recent reports highlighting the influence of nanomaterials features on immune cells response for tissue regeneration, as summarized in Table 2**.**

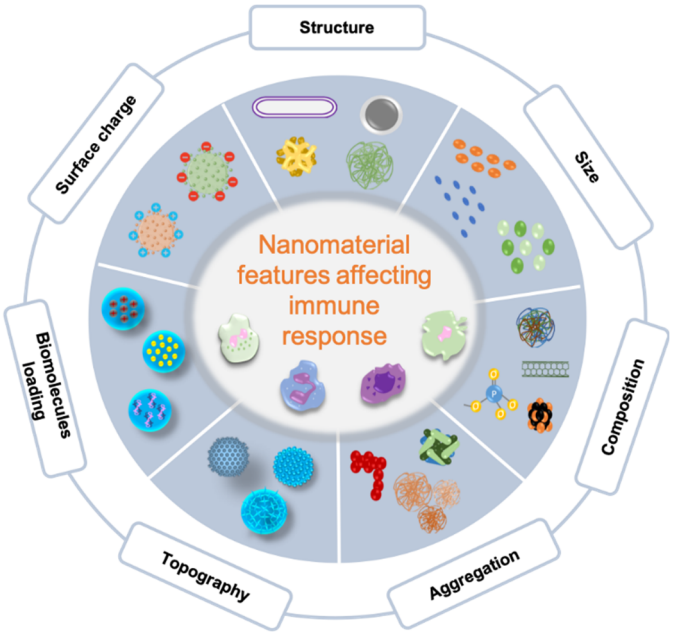

Figure 4. Schematic illustration of the several NMs properties affecting the immunomodulatory bone and cartilage regeneration. The physicochemical, structural and biomolecules loading properties of the NMs play a crucial role in immune cells activity and in their crosstalk with bone and cartilage cells for tissue regeneration.

Table 2. Physicochemical properties of nanomaterials for tissue repair/regeneration.

Nanomaterial Parameters	Properties	Regulatory Effects on Immune Cells	Advantages/ Disadvantages	Application	Ref.
Composition	Heparin methacrylamide nanoparticles	Immune T cells stimulation for cytokines delivery	BMP-2 deliver in controlled doses	Bone regeneration	[121]
	TiO_2 nanotubes	M1-to-M2 macrophages phenotype switch	Co-delivery of RGD peptide and IL-4 MSCs early osteogenic differentiation	Bone regeneration	[120]
	LL-37-loaded SF nanoparticles (SFNPs)-modified Ti samples	Enhanced paracrine signalling between MSCs and macrophages	Superior anti-inflammatory response Improved bone tissue formation in vivo	Bone regeneration	[125]
	Nano-HA-coated microporous Ti surfaces	Inflammation suppression by macrophages activation and polarization to M2 phenotype.	Improved osteoimmune microenvironment Activation of key signaling pathways, TGF-β, OPG/RANKL, and VEGF	Bone remodeling	[124]
	Anti-TNFα Abs-CS/PAMAM dendrimers	Pro-inflammatory phenotype of monocytes Enhanced activity between monocytes and human chondrocytes	Higher TNFα capture when coupled to the CS/PAMAM dendrimers Good anti-inflammatory nanocarriers	Rheumatoid arthritis treatment and modeling	[148,149]
Size	CNT-coated nanofibers (500 nm nanofibers and 25 nm nanotubes)	Macrophages recruitment and activation to accelerate bone tissue regeneration	Up-regulation of osteogenic markers, BMP-2, OPN, OCN	Bone tissue healing	[150]
Biomolecules loading	Mesoporous silica nanoparticles (30 nm porosity)	Polarization of macrophages to anti-inflammatory M2 cells	Higher loading and delivery of IL-4	Targeted delivery of bioactive molecules	[151]

Table 2. *Cont.*

Nanomaterial Parameters	Properties	Regulatory Effects on Immune Cells	Advantages/ Disadvantages	Application	Ref.
Structure	RGD-rich gelatin-coated 1D MnO_2 nanotubes	Anti-inflammatory effects from TGF-β-MnO_2 nanotubes	Strong binding capacity to ECM proteins Favorable physical microenvironment for BMSCs chondrogenic differentiation	Cartilage repair	[152]
Surface chemistry	FP-AuNPs-encapsulated liposomes	Regulation of overproduction and overexpression of immune T cells	FP increased the hydrophilicity of the AuNPs FP-AuNPs increased the hydrophobicity of liposomes Sustained release of FP protein with anti-osteoarthritic and anti-inflammatory effects	Osteoarthritis	[153]
	Ag nanoparticle-loaded TiO_2 nanotubes	Macrophage polarization towards the M2 phenotype	Controlled release of ultra-low-dose Ag+ ions Suitable osteo-immune microenvironment	Bone healing	[154]
Topography	Ti-surface modified micro-nano fibre-like structures	Stimulation of M2 phenotype	Elevated roughness and hydrophilicity promoting a favourable osteoimmune microenvironment Stimulation of osteogenic and angiogenic differentiation	Bone regeneration	[155]
	Cu-modified Ti nano-topographical substrates	M2 macrophages polarization	Upregulation of the TLR signalling Anti-inflammatory properties Osteogenic differentiation	Bone healing and regeneration	[156]
Cell-membrane coating	BMSCs membrane-covered KGN-encapsulated Fe_3O_4 nanoparticles	Controlled immune response promoted by macrophages	Upgraded biocompatibility Higher chondrogenic regeneration capabilities	Cartilage regeneration	[138]
	Neutrophil membrane-coated PLGA nanoparticles	Controlled inflammation levels in the disease progress	Direct use of membranes from the effector cells of the disease Neutralization of relevant inflammatory factors by target specificity Evolution of the existing anti-cytokine agents to a direct function-driven disease blocker	Rheumatoid arthritis management	[139]
	Macrophage macrovesicles-coated (PLGA) nanoparticles	Inflammation reduction, stabilization of arthritis index and suppression of disease severity	Similar bioactivity to that of pure RA-targeting macrophages Bind to HUVECs and targeting arthritic tissue in a mouse model	Rheumatoid arthritis therapy	[139]

Table 2. *Cont.*

Nanomaterial Parameters	Properties	Regulatory Effects on Immune Cells	Advantages/ Disadvantages	Application	Ref.
Protein aggregation	BSF-immersed magnetic nanoparticles	Reduced inflammatory response	Protein corona layer formation induced molecular changes in OA	Osteoarthritis	[157]
	Synovial fluid-incubated PLGA/PS nanoparticles	Modulation of nanoparticles uptake by synoviocytes	Protein corona changed the colloidal stability of nanoparticles in vitro Affected retention in cartilage and arthritic tissue	Osteoarthritis	[158]
	Human plasma-incubated polymeric nanoparticles	Protein corona-bearing nanoparticles increased the activity of human macrophages	Protein corona layer increased hydrodynamic diameters of nanoparticles and changed surface charges Increased release of IL-1ß, IL-6, and IL-10 cytokines	Drug release and controlled inflammation	[159]

MnO_2: Manganese dioxide; Fe_3O_4: Iron oxide; TGF-β: Transforming growth factor-β; OPG: Osteoprotegin; RANKL: Receptor activator of nuclear factor kappa-B ligand; VEGF: Vascular endothelial growth factor; RGD: Arginylglycylaspartic acid; BMP-2: Bone morphogenic protein-2; OPN: Osteopontin; OCN: Osteocalcin; TLR: Toll-like receptor; IL-1ß: Interloukin-1ß, IL-6: Interleukin-6, and IL-10: Interleukin-10; SF: Silk fibroin; TiO_2: Titanium dioxide; HA: Hydroxyapatite; CS: chondroitin sulphate; PAMAM: poly(amidoamine); CNT: Carbon nanotubes; 1D: One-dimensional; ECM: Extracellular Matrix; BMSCs: Bone marrow-derived mesenchymal stem cells; FP: Fish oil protein; AuNPs: Gold nanoparticles; Ag: Silver; PLGA: poly(lactic-co-glycolic acid); HUVECs: Human umbilical vein endothelial cells; RA: Rheumatoid arthritis; BSF: Bovine synovial fluid; OA: Osteoarthritis; PS: Polystyrene.

Composition

Nanomaterials for bone regeneration can be sourced from a variety of raw materials, including polymeric, ceramic, carbonic or inorganic (e.g., metal and silica) [113,114], and these raw materials can regulate the immune response. Especially, several metal elements derived from nanomaterials, such as Zn, Sr, Eu, and Au, have been shown to play roles in immunomodulation.

As mentioned in 4.1.1.1, Zn^{2+} from IL@ZIF (zeolitic imidazolate frameworks) nano-platform was found to inhibit macrophage inflammatory response by reducing ROS accumulation, thereby benefiting tissue regeneration [122]. By the same token, Li et al., designed a programmed local delivery system based on a micro-nano surface of titanium [160]. Titanium surface was treated with a heat-treatment and posteriori combined with poly-dopamine to construct AH-Sr-AgNP structure with the ability to release Ag^{+} and Sr^{2+}, which facilitating osteogenesis both in vitro and in vivo by inducing antibacterial effects as well as M2 macrophage polarization [160]. Another study demonstrated that Europium-doped mesoporous silica nanospheres (Eu-MSNs) were a great immunomodulatory tool for inducing osteogenesis and angiogenesis [161]. Europium can act as calcium to stimulate bone regeneration process. Researchers found that compared with MSNs, macrophage stimulated by Eu-MSNs secreted factors to induce osteogenesis and angiogenesis of BMSCs and HUVECs, respectively. Further, the induced bone regeneration in vivo following Eu-MSN treatment suggests that the modulated immune microenvironment by Eu-MSNs should benefit bone healing [161]. In the same strain, Liang et al., used mesoporous silica nanoparticles loaded with gold (Au-MSNs) to enable osteogenesis via immunomodulation [162]. Au-MSNs directed a M1-to-M2 conversion in macrophages, which allowing for an ideal environment for bone regeneration in vitro and in vivo [162].

The potential of dendrimers as immunomodulatory and anti-inflammatory nanoparticles have also been demonstrated [163]. Different types of dendrimers have been developed using diverse materials capable of modulating the dendrimer's core, branches and functional terminal groups that ultimately tailor their properties for achieving immunomodulatory features and specific cell targeting. Recently, Oliveira et al. developed poly(amidoamine) dendrimers (PAMAM) functionalized with chondroitin sulphate (CS) and anti-TNFα antibodies (Abs) as anti-inflammatory promoters in rheumatoid arthritis [148]. The proposed anti-TNF-α Abs-CS/PAMAM dendrimers showed higher capacity for TNF-α capture when coupled to the CS/PAMAM dendrimers which could be useful for controlled drug delivery and more effective anti-inflammatory activity. The in vitro studies, also revealed that the CS/PAMAM dendrimer nanoparticles presented cytocompatibility and hemocompatibility

properties. The therapeutic anti-inflammatory efficacy of the anti-TNFα Abs-CS/PAMAM dendrimers were also tested using a human 3D inflammatory cartilage-on-a-chip model by loading the nanoparticles in Tyramine-Gellan gum hydrogels [149]. The therapeutic approach was validated using a human macrophage cell line (THP-1) and human chondrogenic primary cells (hCH) cells simulating the anti-inflammatory system. After 14 days of in vitro culture, anti-inflammatory effects were observed and the inflamed hCH presented high expression of collagen type II, indicating that the cells were able to recover and maintain their biological functions. Thus, authors were able to simultaneously demonstrate the efficacy of the anti-TNFα Abs-CS/PAMAM dendrimers as anti-inflammatory nanocarriers (against macrophage inflammatory response to improve hCH function) and the Tyramine-Gellan gum hydrogels as potential preclinical in vitro models of rheumatoid arthritis.

Size and Structure

The size and shape of nanomaterials have a significant impact on their uptake by host and immune cells, as well as, on the triggering of the immune response and their overall bio-distribution in vivo [141]. Nanomaterials can be divided into one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D). The above-mentioned nanoparticles, nanospheres, nanofibers, nanotubes and nanorods are 1D nanostructures presenting a nanoscale size in special dimension [164]. The 2D nanostructures include nanoflakes and nanoporous microstructures, whereas the combination between 2D and 1D nanoarchitectures forms the 3D hierarchical structure of nanomaterials, which are beneficial for bone regeneration. Increasing reports indicate that the 1D level of nanomaterials is more beneficial to activate and regulate the intrinsic immunomodulatory response and enhance tissue regeneration capacity [165]. Simultaneously, the shape of these 1D nanostructures also affects the host and immune cell uptake and response. Reports have indicated that rod-shaped nanoparticles with a larger surface area than spherical nanoparticles were much more likely to be taken by macrophages. This was attributed mainly to the generated vesicles, which allow for easier engulfment via micropinocytosis. For rod-shaped nanomaterials, the aspect ratio was reported to govern cytokine secretion; nanoparticle with larger aspect ratio can lead immune cells to produce more inflammatory cytokines like IL-6 and IFN- γ [147], thus enhancing their inflammatory responses.

In a recent review, the effects of size and shape on gold nanoparticles (AuNP) on the endocytosis and immune response of macrophage have been summarized [166]. In general, spherical AuNPs (sAuNPs) are more anti-inflammatory than rod-shaped AuNPs (rAuNPs), and the anti-inflammatory effect is negatively correlated with the aspect ratio of AuNPs [166–168]. In sAuNPs, the effects of size on NP uptake and macrophage response differ between the naked and surface modified AuNPs. For the naked sAuNPs, the uptake and anti-inflammatory effect increase along with size decreases, whereas for the sAuNPs with surface modification, the uptake and anti-inflammatory effect decrease along with size decreases [166,169,170]. The uptake efficiency in macrophage is highly associated with AuNP shape [166–168]. The uptake of rAuNPs is stronger than that of sAuNPs [166–168]. Meanwhile, the uptake of triangle shaped AuNPs (tAuNPs) is much more difficult than that of sAuNPs [166,171]. Interestingly, the uptake of tAuNPs increases with NP-size (determined by triangle-length), whereas the uptake of sAuNPs decreases with NP-size (determined by sphere-diameter) [166,168]. This phenomenon is attributed to the initial stage of endocytosis process, which consisting of NP adsorption on cell membrane, and the sequential wrapping of NP by cell membrane which starts at the NP region with high-rate curvature (the rate at which a curve changes direction in relation to the distance along the curve) [166,168]. For tAuNPs, the NPs with larger size obtain a larger contact surface area facilitates NP adhesion with the cell membrane, and membrane wrapping starts at the triangle edge regions (a region with high-rate curvature) which is not affected by triangle size. On the other hand, the uptake of sAuNPs is highly determined by NP size, that smaller NP with higher curvature can be more easily internalized by cell [166,168].

The 2D nanoscale topographical surfaces play a crucial role in bone tissue repair and regeneration. Nanoporous microstructures with different sized pores may present distinct modulatory effects on the osteo/chondro-immune response of macrophages [172]. Researchers found that nanopores with different sizes and structures are critical to the morphological changes of macrophages and host osteogenic cells, suggesting that the surface roughness and nanotopography can modulate the immune microenvironment for better tissue repair. For example, in our previous work [173], mesoporous silica rods were found to reduce macrophage inflammatory response, as compared with smooth silica rods, suggesting the surface structure of nanoporous materials can affect immune response.

It is well recognized that the 3D nanoarchitecture of biomaterials is critical to mimic the hierarchy of bone tissues for improved regeneration strategies. As example, tailoring the surface of polymer nanofibers with carbon nanotubes has been shown to create a bi-modal nanoscale surface topography (500 nm nanofibers and 25 nm nanotubes) for modulating immune cells response, angiogenesis and bone regeneration [150] (Figure 5B). The

CNT-coated nanofibers significantly reduced inflammatory signals and substantially promoted angiogenic response stimulating bone tissue healing by the up-regulation of osteogenic markers (BMP-2, OPN, OCN). In a different study, injectable hybrid inorganic (IHI) nanoscaffolds were produced by coating 1D MnO_2 nanotubes with L-Arginyl-Glycyl-L-Aspartic acid (RGD)-rich gelatin and used as template for assembling stem cells in a chondrogenic differentiation strategy [152]. The 1D MnO_2 nanotubes were chosen based on their high surface area for pro-chondrogenic factors loading and to mimic the 1D collagen fibrils performance in the cartilage ECM. Thus, the IHI nanoscaffolds strongly bind to the ECM proteins assembled in stem cells forming a 3D cell-cell and cell-ECM interactions favorable to chondrogenic differentiation. Moreover, the IHI nanoscaffolds are capable of suppressing inflammatory microenvironment by the unique MnO_2 composition. Additionally, the biomimetic hierarchical intrafibrillarly mineralized collagen scaffold (as mentioned in Section 4.1.1) has been found to favor M2 macrophage polarization due to its bone-like staggered nanointerface [119], suggesting that the biomimetic bone structure can be utilized for nanoengineering of immunomodulatory materials. In general, nanoparticle size, shape and structure can affect the responses of immune cells towards these particles, whereas the immune cell responses in different manner towards different particle type (composition, shape, surface structure and/or modification), it is hardly possible to summarize the ideal size/shape/structure for certain nanoparticle type because of the lack of studies to dig out the fundamental cellular and molecular mechanisms underlying the nanoparticle-immune cell interplay, which suggesting future studies to address this knowledge gap.

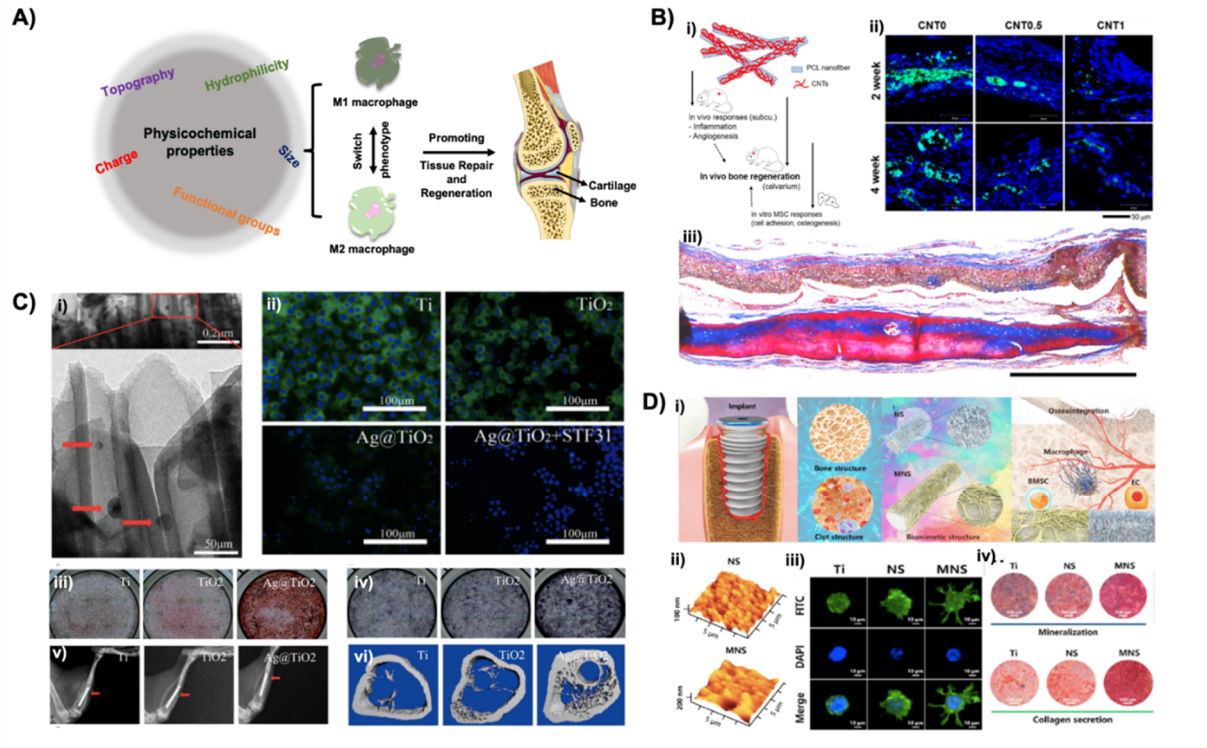

Figure 5. (**A**) Schematic illustration of the different nanomaterial surface properties capable of affecting the immune behaviour of macrophages and tissue repair and regeneration. (**B**) CNT-coated PCL nanofibers with unique bi-modal nanoscale topography (500 nm nanofiber with 25 nm nanotubes) for inflammation, angiogenesis, and bone regeneration. (**i**) Schematic illustration of the of the in vitro and in vivo biological assays performed with CNT-coated PCL nanofibers. (**ii**) Expression of pro-inflammatory signals of pan-macrophage marker F4/80. (**iii**) Bone regeneration analysis revealing a matured new bone structure. Adapted with permission from [150]. (**C**) Surface modified antibacterial silver nanoparticle-loaded TiO_2 nanotubes (Ag@TiO_2-NTs) and their influence on macrophages polarization and osteoimmune microenvironment. (**i**) TEM images of Ag@TiO_2-NT (red arrows indicate AgNPs). (**ii**) Immunofluorescent staining for glucose transporter 1 (GLUT1) in macrophages cultured on the Ti, TiO_2-NTs and Ag@TiO_2-NTs surfaces, showing a lower GLUT1 expression reflective of anti-inflammatory status on the Ag@TiO_2-NTs group. Osteogenic ability of MC3T3-E1 cells, observed from (**iii**) alizarin red and (**iv**) ALP staining. Healing capacity of bone defects, evaluated by (**v**) X-ray and (**vi**) micro-CT, 2-weeks after surgery. Adapted with permission from [154]. (**D**) Micro/nano-scale (MNS) titania fiber-like network on the surface of titanium (Ti) implants. (**i**) Illustration of the implant and 3D model of the biomimetic structure on the Ti implants with the de novo bone formation microenvironment during the osseointegration. (**ii**) Surface roughness of the

surfaces obtained by AFM. (**iii**) Osteoimmunomodulation of M1 macrophages on the biomimetic surfaces. (**iv**) Mineralization and collagen type I secreted by BMSCs after osteogenic induction for 7 days on the biomimetic surfaces. Adapted with permission from [155].

Surface Properties

The surface properties of nanomaterials are capable of triggering sequential foreign body reactions including nonspecific protein adsorptions and macrophages polarization [174]. Various surface features, such us, the type of functional groups, changes in hydrophilicity, surface charge, and topography of nanomaterials have a critical role in modulating immune cell responses and affecting bone tissues repair and regeneration (Figure 5A). Nanomaterials with hydrophilic functional groups have been reported to induce M2 macrophages activation, thus improving bone tissue healing and the production of osteogenic cytokines responsible for the superior osteointegration of implanted materials [175]. Fish oil protein (FP) tagged with AuNPs and further encapsulated in liposomes, a strategy to increase the hydrophilicity of the nanoparticles and liposomes [153]. The FP-AuNPs-encapsulated liposomes showed a sustained release of the adsorbed FP protein in simulated body fluid, and when injected into intra-articular joints of rats, the FP-AuNPs-encapsulated liposomes displayed anti-osteoarthritic effects inhibiting inflammatory immune response. Therefore, the surface-modified hydrophilic nanomaterials could enhance osteointegration by promoting M2 macrophage polarization. Previous studies [114,154] also demonstrated the modification of nanomaterials with a variety of functional groups, e.g., phosphonic acid, amide, carbon, nitrogen or oxygen, affected the surface charge of nanomaterials and modulated the macrophage polarization between M1 and M2 phenotype and by extension the tissue healing capacity (Figure 5C).

In addition to surface chemical properties, the physical surface properties such us topography and roughness can also modulate cell performance. This is transversal to immune cells like macrophages, whose phenotype and polarization can change according to nanomaterial topography, and to bone-forming cells by changing their shape and elasticity. As example, Bai et al. [155] performed surface modification on pure Ti to obtain nano and biomimetic micro-nano fibre-like structures and investigated the effect of different surfaces on osteo-/angio-genesis and osteoimmunomodulation. It was observed that the micro/nano biomimetic coating induced elevated roughness and hydrophilicity, promoting a favourable osteoimmune microenvironment by stimulating the M2-like phenotype. Moreover, the osteogenic differentiation of BMSCs and angiogenic differentiation of endothelial cells were stimulated, improving the multi-signalling pathways and crosstalk between osteogenesis and angiogenesis. Osteointegration was found to be ameliorated when induced by micro-nano topography as compared to the single nano-fibre structure as well as the pristine Ti implant, which was further confirmed in vivo (Figure 5D). In another work, micro-Ti substrates were nano-topographically modified using Cu-coating to explore the role of Cu^{2+} release in regulating macrophages polarization and macrophages-mediated osteogenesis [156]. The Cu-modified Ti nano-topographical substrates displayed an anti-inflammatory role by upregulating the TLR signalling. Moreover, a favourable immune microenvironment was achieved for osteoblasts proliferation and differentiation. Recently, the nanoscale roughness of materials have been recognized to also affect osteoblasts performance induced by immune cells activity [113]. Li et al. [176], analysed the effects of nano-scaled Ti surface roughness (100–400 nm) on osteoblasts and macrophages response, showing that osteoblasts differentiation was favoured by the increase in Ti substrates surface roughness, affected by the macrophages activity that showed a tendency to polarize toward M1 phenotype with increased levels of TNF-α, IL-6, IL-4 and IL-10 production. Thus, increasing surface roughness of nanomaterials favors osteoblastic activity by modulating immune cells response.

Protein Aggregation Properties

When nanomaterials invade biological environment, their surface tends to be coated by proteins, forming the "protein corona". This is an inevitable process, which gives to nanomaterials new functional, physicochemical and biological surface properties [177]. In fact, several studies reported the numerous factors that contribute to the type of protein corona layer formed on nanomaterial surface, including their size, shape, and charge. Nanomaterials with hydrophobic or highly charged surfaces typically adsorb more proteins than those with hydrophilic or neutral surfaces [178]. At the same time, nanomaterials with larger surface area provide larger contact areas with proteins and thus more nonspecific interactions with serum proteins being advantageous for their adsorption and corona formation [179]. The spherical-shaped nanomaterials have also shown to induce higher protein adsorption with thicker corona formation than the irregular-shaped ones [180].

Regarding protein corona effects on immune cells response and pathological conditions, it has been observed that corona works as a new biological identity of nanomaterials capable of activate and differentiate different cell types for a desired biological response. Shah et al. [157], proposed the formation of protein corona by immersing

commercial magnetic nanoparticles in bovine synovial fluid followed by testing in osteoarthritic rat models (injecting the particles into the animal osteoarthritis (OA) joints). The in vivo results demonstrated that the protein corona formed on the surface of the nanoparticles varied according to the stage of OA progression, affecting immune cells activation, differentiation and activity. The interactions between nanomaterials and synovial fluid have been discussed as disadvantageous in cartilage tissue, by increasing the size and shape of nanoparticles which hinder their uptake by the cells at the OA area thus impeding the efficacy of drug delivery into these cells [158]. Obst et al. [159] developed protein corona on six polymeric nanoparticles incubated with human plasma, and showed that the corona bearing nanoparticles presented different cellular adhesion and surface charge according to the shape of the nanoparticles and formed corona layers. Moreover, a marked increase of IL-1ß, IL-6, and IL-10 release from primary macrophages was observed [159]. In a different study [181], human monocytes polarized into M1 and M2 macrophages had different internalization patterns from silica nanoparticles pre-immobilized with human serum. The M2 cells showed a higher internalization of the protein corona formed at nanoparticles surface, whereas the M1 cells were more interactive with the pure nanoparticles. Thus, these studies confirm that the protein aggregation properties and corona layers formation at nanomaterial surface can be decisive for guiding immune cells fate and activity in disease modulation, including bone and cartilage complex diseases.

In brief, the emerging knowledge about nanomaterial effects on immunological response and consequent bone tissues repair/regeneration, allowed researchers to focus on nanotechnology for the design of "smart" biomedical-based nanomaterials capable of simultaneously targeting immune cells, tissue cells response and drug delivery. Meanwhile, the understanding of nanomaterial-immune system interactions is critical for developing more effective biomaterials for osteoimmunomodulation and tissue regeneration, which should not be ignored in nanomaterial design. Nevertheless, the big challenge is to control the physicochemical parameters of nanomaterials that can modulate immune response and affect highly complex molecular networks interacting with the skeletal and cartilage tissues. For that, researchers are continuing to pursue optimal fabrication techniques to tune the ultimate physicochemical and structural surface properties of nanomaterials for the precise modulation of bone homeostasis. Finally, it is important to emphasize the potentially adverse reactions of nanomaterials to the surrounding in vivo tissues. Side effects of cytotoxicity, inflammation and undesirable organ targeting are still possible, and much room remains to optimize in this sense. Computational modelling and artificial intelligence are poised to revolutionize nanoresearch by providing systematic optimization steps for nanotechnologies, predicting immune reactions, and facilitating the design of nanomaterials for improved immunotherapies.

4.2. Multi-Scale Design and Modification of Bone Implant Material

Immune reactions play a crucial role in determining the in vivo fate of bone implant materials. Once the host recognizes the implants, the immune cells are immediately triggered to release cytokines and chemokines. The regulatory molecules will lead to persistent excessive inflammation or conversely contribute to the efficacy of regeneration [182]. The rise of osteoimmunology highlights the importance of considering immune system responses in implant design. A favorable immune microenvironment mediated by biomaterials is critical to the process of tissue regeneration. This chapter mainly focuses on the potential of utilizing the physicochemical properties of biomaterials, including surface property, structure, modification, material porosity, and released ions (Table 3), for osteoimmunomodulation.

4.2.1. Surface Property and Modification

The surface physiochemical properties of implant material, including surface topography, hydrophilicity, roughness, and elasticity modulus, are critical contributors to the local osteoimmune environment.

Surface Structure

Owing to the immunomodulatory potential of nanosurface (as discussed in Section 4.1.2), designing the surface nanostructure of implants has become a viable strategy to confer osteoimmunomodulatory properties to implant materials [183]. Previous studies have found surface topography, especially nanotopography can influence macrophage immune response. In our previous study, porous anodic alumina surface with pore size ranged from 0 to 200 nm was used to stimulate macrophages. The results showed that macrophages exhibited different morphologies and reduced expression of inflammatory markers as nanopore size increased [172]. Similarly in another study, a group of hydroxyapatite bioceramics with hierarchical surfaces were prepared, which consist of 4/12/36 μm microdots containing nanoneedles. Macrophages cultured on these surfaces displayed different morphology along with the distribution of nanoneedles, and expressed lower levels of M1 markers while higher levels of M2 markers along with the size increase in microdots [184].

Beside pore size, the morphology of surface nanostructure can affect immune response. In a recent study, a group of PFCH (poly (lactate-co-glycolate)/fish collagen/nano-hydroxyapatite) fibrous membranes were synthesized with random, aligned and latticed topographies. Membranes with latticed topographies showed superior macrophage recruitment and M2 polarization, which leading to improved bone regeneration, as compared to the other two topographies [185]. Similarly, another study compared the macrophage responses to nano-concave pit (NCPit) and nano-convex dot (NCDot) microarrays, and the findings revealed that NCDot could induce macrophages to polarize toward the M2 phenotype, resulting in a favorable osteoimmune microenvironment to promote osteogenesis (Figure 6) [186]. Although the detailed mechanism underlying nanotopography -associated immunomodulation is not clear, considering the fact that the surface with different-sized nanopores or different-shaped morphology can influence cell attachment and spreading, these finding suggesting that nanotopography may affect macrophage response via biophysical signals which are yet to be explored.

Interestingly in another study, Zheng et al. designed a dynamic surface topography which can transform from flat to microgroove when stimulated by NIR irradiation. The NIR-triggered surface switch changed macrophage morphology from round to elongated shape (the average elongation factor of macrophages increased by about 5 times), with upregulated expressions of M2 makers (Arg-1 and IL-10) in vitro and induced M2-like macrophage polarization in vivo, indicating the immunomodulatory effect based on the close relationships between the phenotype and macrophage morphology change induced by mechanical force-stimulated reorganization of cell cytoskeletons [187]. However, there are controversial results, as previous studies suggest that round-shaped macrophage is M2-like, and elongated macrophage is M1-like [172,184], while the mechanical force-induced morphology transformation from round to elongated shape induces a M1-to-M2 phenotype change [187]. Although the detailed mechanisms underlying nanotopography-associated immunomodulation are not fully understood, the fact that surfaces with different-sized nanopores or different-shaped morphologies can influence cell attachment and spreading suggests that nanotopography may affect macrophage response through biophysical signals. However, these mechanisms remain to be explored further

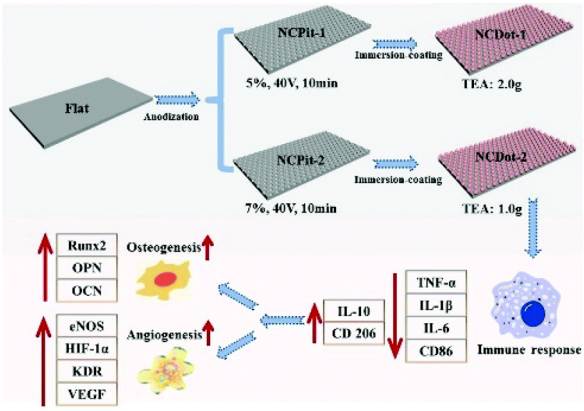

Figure 6. The prepared NCDot arrays were able to significantly promote osteo-/angiogenic activity by generating a more suitable immune microenvironment than the corresponding NCPit arrays. Reproduced from [186].

Hydrophobicity and Surface Roughness

Although the detailed mechanism for surface structure associated immunomodulation is not clear, data have shown that surface topography can change hydrophilicity, a significant determinant in osteoimmunology by influencing immune cell response and cell attachment to the implant [188]. Previous studies have found that nanotopography can change the surface hydrophilicity, and compared with hydrophobic nanostrucuture (e.g., anodic alumina surface with small pore size (0–15 nm), NCPit), the hydrophilic one (e.g., anodic alumina surface with large pore size (50–200 nm), NCDot) can reduce the M1 polarization [186] (Figure 6). Generally, hydrophobic surface can adsorb more protein in comparison to hydrophilic surface [189]. Meanwhile, along with the increase in hydrophilicity, the adherence and M2 polarization of macrophage (or macrophage precursors) are increased [188,190], and further investigations suggest the roles of adsorbed proteins (fibronectin and fibrinogen), cell-binding cites, and integrins in hydrophilicity associated immunomodulation. Compared with hydrophobic surface, hydrophilic titanium surface was found to provide more fibronectin-binding sites per cell, and the interaction between fibronectin and integrin β1 on cell surface can direct the M1-to-M2 phenotype switch via activation on the phosphoinositide 3-kinase and serine/threonine kinase Akt (PI3K-Akt) signaling pathway, then sequentially inhibiting NF-κB, a typical signaling pathway in M1 polarization (as explained in the previous

sections). By contrast, hydrophobic surface provided more fibrinogen-binding cites per cell, then facilitated a M1 polarization via interaction between fibronectin and integrin β2 to activate NF-κB [190]. Similarly, another study demonstrated that compared with the smooth and hydrophobic surface, a rough hydrophilic titanium surfaces may facilitate macrophage polarization toward an anti-inflammatory M2-like phenotype, as evidenced by increased IL-4 and IL-10 production [191], suggesting surface roughness may exert an immunomodulatory effect. In addition to the above surface properties, another study found that the elastic modulus of the material surface played a vital role in regulating immune cell behavior [192], that surface with higher elastic modulus was found to favor an anti-inflammatory M2-like phenotype via inhibiting NF-κB. These findings underscore the importance of controlling surface properties to modulate immune responses and promote favorable outcomes in bone tissue regeneration.

Modification Approaches

The immunoregulatory effects of surface properties have attracted investigations on surface modification approaches for functional osteoimmunomodulation [188]. To date, chemical, physical, electrochemical, and biochemical approaches are commonly used to coat a thin layer for the implant surface. Our previous study showed that a barrier collagen membrane coated $Ca_2ZnSi_2O_7$ ceramic via PLD (pulsed laser deposition) technique optimized the osteoimmunomodulatory property, which effectively improved the osteogenic differentiation of BMSCs [193]. Our further study found that the prepared SMS ($Sr_2MgSi_2O_7$) coatings directed macrophage polarization from M1 to M2, which hampered the inflammatory reaction through the inhibition of TLR(Toll-like receptor) and Wnt5A/Ca^{2+} pathways of macrophages [194]. Bai. et al. designed a TiO_2 coating decorated with hydroxyapatite nanoparticles which was generated by micro-arc oxidation (MAO) of pure titanium and followed with annealing, in which MAO-650 (at an annealing temperature of 650 °C) not only supported the proliferation and differentiation of osteoblasts, but also inhibited the inflammatory response of macrophages and enabled a favorable osteoimmunomodulation to facilitate osteogenesis [195]. The osteoimmunomodulatory effects of these coatings could be due to the functional ions (such as Ca, Si and Mg ions), however, further studies are suggested to investigate the associated biomechanisms (e.g., biomolecules, signaling pathways) underlying the regulations of surface coating on immune cells and osteoblasts, which shall generate critical knowledge to guide the future material design.

4.2.2. Porosity of Biomaterials

The effect of porosity on immune cell response has been extensively investigated [196,197]. Higher porosity is thought to be beneficial for osteogenesis. In addition to affecting the behavior of bone cells, the importance of porosity is reflected in the interaction between the implant and the host immune cells [198–200]. The porous methacrylate/silica hybrid scaffold with 60.5 ± 1.1% percent porosity instigated new vascularized bone formation, and showed preferable M1/M2 macrophage profile [201], although the detailed mechanisms underlying this phenomenon should be further explored Similarly in another study, macrophages cells were cultured on biomimetic calcium deficient hydroxyapatite (CDHA) substrates with different porosity (ranging from 34.3% to 54.4%). The findings revealed that samples with higher porosity (54.4%) induced the expression of molecules include OSM, TGFβ1 and VEGF in macrophages [182] which can improve osteogenesis by facilitating osteoblast differentiation. However, currently it is unclear how material porosity leads to the changes in macrophage growth factor release, and future studies are suggested to explore the mechanisms underlying material porosity associated immunomodulation. Additionally, although higher porosity is better for osteoimmunomodulation, porosity should be controlled at certain level to avoid the adverse effects of high porosity, such as the risk of implant failure or complications related to excessive inflammation or infection. Also, it would be beneficial to explore how porosity interacts with other surface properties to regulate immune cell behavior and osteogenesis.

4.2.3. Released Ions

In the process of gradual degradation, the chemical ions released from the implant material can cause significant effects by changing the microenvironment [202], which thereby regulating the local immune cells (e.g., macrophage polarization) to create an immune environment to favor osteogenesis. For example, europium ion (Eu^{3+}, 0.066 ± 0.035 μg/mL) stimulated macrophages to generate appropriate immune response through secreting more pro-inflammatory cytokines and further promote osteogenic differentiation of BMSCs by upregulating their expression of osteogenic genes of COL-I, ALP, OPN and OCN [161]. Strontium ion (Sr^{2+}) at a concentration of 1.30 μg/mL exhibited a significant effect on BMSCs osteogenic and chondrogenic differentiation in vitro and simultaneous regeneration of cartilage and subchondral bone in vivo by switching the macrophage into M2-like phenotype [203], via modulating NF-κB activation [204]. Magnesium ion (Mg^{2+}) released from magnesium-

containing microspheres cement extracts with concentration = 3.125 mg/mL was demonstrated to be an affective anti-inflammatory agent. Mg was introduced into bone cement materials, and findings revealed that it triggered positive immunomodulation through upregulation of the anti-inflammatory IL-10 and M2 polarization of macrophage with higher expression of CD206 [205]. Copper ion (Cu^{2+}, 1.523 μg/mL) appeared to have a positive effect on osteogenesis via the activation on macrophages to secrete OSM as shown in Figure 7 [206]. OSM then activates the OSM pathway (a pathway associated with osteogenesis [50]) to activate osteoblast differentiation, thereby promoting osteogenesis [206]. Another study showed that copper ion (Cu^{2+}, 0.50 ~ 16.02 μg/mL) stimulated macrophages to secret more anti-inflammatory cytokines and further facilitated the proliferation and maturation of chondrocytes [207]. Interestingly, cobalt (Co^{2+}, 0.260 ± 0.035 μg/mL), zinc (Zn^{2+}, 0.160 μg/mL), manganese (Mn^{2+}, 1.485 ± 0.129 μg/mL) ions have also been shown to hold immunomodulatory function (which functionally regulate anti-inflammatory M2 macrophage polarization) and thus benefiting bone regeneration [208–211]. Although detailed mechanisms are unclear, it should be noted that the response of the immune system is closely related to the concentration and release rate of these bioactive ions. Thus, further studies are suggested to find out the ideal dose and release rate of certain ions for immunomodulation. In addition, the precise control of implant ion behavior should be further investigated. Although some studies have initially explored the potential mechanism between ions and immune responses, future study is required to demonstrate the molecular mechanism, so as to promote the further development of bone implant materials to release chemical ions with functional osteoimmunomodulatory effect.

Obviously, the immunomodulatory effects of materials can be adjusted from the characteristics of different dimensions, but these factors are often overlapped. For example, surface properties and released ions [208], surface properties and structural design [212], porosity and structural design [172] and so on. It is interesting that no matter what characteristics of the material changed, it shows a very universal phenomenon: certain properties of bone implant materials affect the quantitative distribution of the two phenotypes of macrophages to form a favorable bone immune environment, most of which are polarized into anti-inflammatory M2 phenotypes. However, the specific mechanism still needs more extensive and in-depth research to prove. In short, from the point of view of material design, it is indeed possible to obtain an almost ideal bone implant. Whether the commonality or internal connection between these phenomena can be found is a question that needs to be considered in the next generation of bone biomaterials.

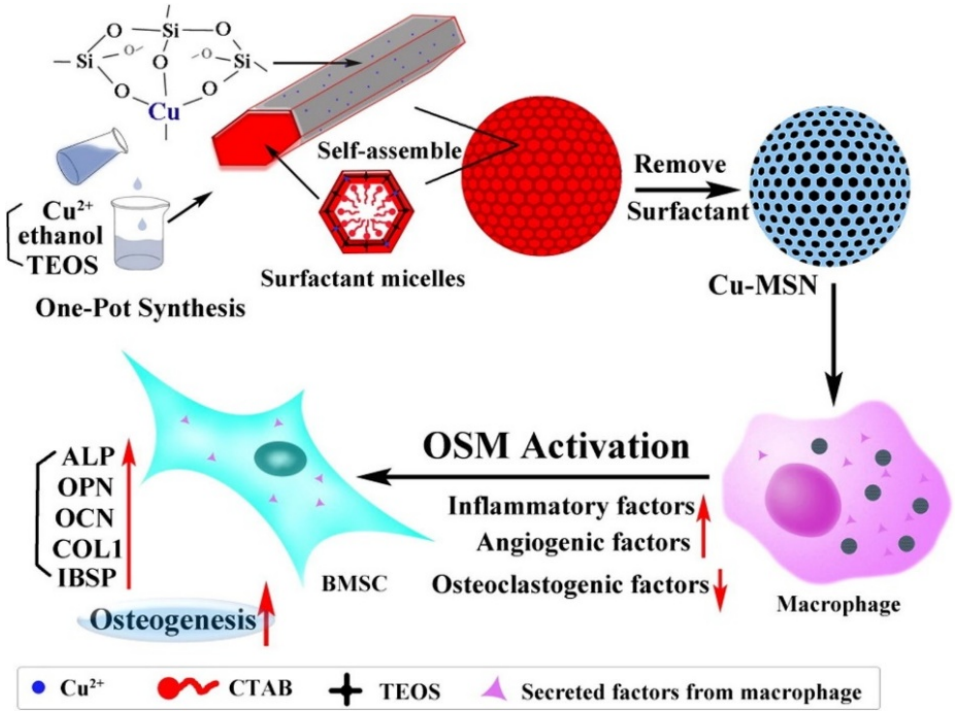

Figure 7. The incorporation of Cu^{2+} into mesoporous silica nanospheres (Cu-MSN) induced beneficial immune response and further stimulated the osteogenic differentiation of BMSCs. Reproduced from [206].

Table 3. Effects of material design and modification approaches on immune cell responses.

Material Design or Modification Approaches	Effects on Immune Cells Responses
Structural design	Hierarchical, porous or topological structure enhance angiogenesis through inhibiting inflammatory response [172,182,184].
Surface properties	Elastic moduli, topography, hydrophilicity of biomaterials surface modulate macrophage phenotype and promote osteogenesis [187,192,212–214].
Porosity	Higher porosity is in favor of ingrowth of vessel tissues, preferable M1/M2 macrophage profile and enhancement of secretion osteogenic molecules [182,198,199,201].
Released ions	Bioactive ions released from implant materials hold immunoregulatory effects in a concentration- dependent manner [161,203–211]
Coating modification	Implant materials coated bioactive layer by pulsed laser deposition, micro-arc oxidation, plasma-spray et al. methods promote the polarization of macrophages to M2, offering a favorable osteoimmunomodulation [193–195].

5. Current Approaches to Target Cellular Senescence

As discussed in Section 3.1, the indispensable roles of ROS in both cellular senescence suggest that ROS-scavenge should be an efficient approach for inflammaging control. Recently with the development of nanotechnology, numerous high-efficient ROS scavenging nanomaterials with superior stability, enhanced anti-oxidative ability, and biocompatibility have been prepared to protect cells from oxidative stress.

The ROS-scavenging nanomaterials could be roughly categorized as enzyme-mimicking nanoparticles, free-radical trapper nanoparticles, and redox ROS-scavenging nanoparticles. For instance, Ma et al. fabricated an enzyme-mimicking single-atom catalyst with atomically dispersed Fe-N_4 sites anchored on N-doped porous carbon materials (Fe-SAs/NC) that mimicked the antioxidative enzymes of catalase and superoxide dismutase. The Fe-SAs/NC catalysts exhibit great H_2O_2 and O_2^- eliminating ability, which could efficiently protect cells against oxidative stress [215]. Besides, TEMPO-loaded nanoparticles have also been used in antioxidant applications because of their outstanding free radical scavenging capability. For example, Zhang et al. loaded TEMPO into oxidation-responsive β-cyclodextrin nanoparticles (Tpl/OxbCD NP) to develop a superoxide dismutase (SOD)/catalase (CAT) mimetic nanomedicine [216]. Moreover, redox-based molecules such as curcumin, bilirubin, and polydopamine have been prepared as nanoparticles to remove ROS. For example, Bao et al. prepared polydopamine-based nanoparticles (PDA NPs) as biodegradable ROS scavengers for periodontal disease treatment [217]. PDA NPs showed excellent ROS-scavenging ability in human gingival epithelial cells and macrophages, and could protect cells from inflammation reactions. These ROS-scavenging nanomaterials are therefore considered as promising candidates for inflammaging control, and their applications in aging associated disease treatments (e.g., aging bone healing) should be further explored. Furthermore, these nanomaterials can be functionalized with specific cell-targeting capabilities (using the techniques listed in Section 5.1.1) for different purposes (e.g., regulating senescent macrophage polarization without affecting other cells).

Excessive accumulation and insufficient clearance of senescent cells lead to multiple diseases, tissue dysfunction and organ aging [75]. To achieve adequate senescent cell clearance, researchers has developed several pharmaceutical approaches. For example, Rymut et al. treated accumulated pro-inflammatory senescent cells with resolving D1 (RvD1) [218]. In a hind limb ischemia-reperfusion remote lung injury model, the treatment of RvD1 could effectively mitigate the ischemia-reperfusion lung injury in aging, promoted efferocytosis, and prevented the decrease of MerTK in injured lungs from old mice [218]. Recently, CD9-modified silica nanoparticles have been developed to treat senescence induced atherosclerotic plagues [219]. In this study, CD9 antibody was used to target senescent cell membrane biomarker CD9. The CD9 antibody-modified silica nanoparticles could effectively target senescent cells, reduce the ROS and high-density lipoprotein oxidation level, and attenuate the senescence process. In another study, a cytotoxic drug gemcitabine has been modified into a prodrug called SSK1 to be cleavable by lysosomal β-galactosidase activity [220]. The SSK1 showed strong senolytic activity in mouse embryonic fibroblasts, human embryonic fibroblasts, preadipocytes and HUVECs. Moreover, the SSK1 could selectively eliminated human senescent cells induced by different stresses including replicative stress, hydrogen peroxidase, oncogene activation and irradiation. Similarly, SSK1 was able to clear senescent cells in the liver and kidney of aged mice and dampened the inflammation response [220]. Biomaterials for senescent cell clearance are therefore suggested in future research to benefit the treatments of ageing associated diseases.

Currently the above-mentioned strategies (ROS scavenging and senescent cell clearance) haven't been adopted in bone healing research or clinical applications. This is mostly due to the lack of concept of developing

specific biomaterials to improve bone healing in the aged patient: current biomaterial research mostly focused on designing and testing materials for the young patient, which ignored the drastic differences in osteoimmunology status between the young and old. Considering the difficulties in regulating osteoimmunosenescence, it is suggested to involve anti-senescence and anti-inflammaging functions in future biomaterial design.

6. Future Prospectives

Despite the studies listed above, further biomedical studies are required to provide an ideal resolution to aging associated bone healing problems. Firstly, so far, the immune-skeletal cell interplay under senescence conditions (osteoimmunosenescence) is not clear. Compared to osteoimmunology in young cells, osteoimmunosenescence includes not only a pathological inflammatory condition (inflammaging) to impair osteogenesis, but also a vicious paracrine senescence process to boost senescent cell accumulation, and their insufficient clearance due to dysfunctional senescent immune cells. A further investigation on cellular and molecular mechanisms underlying osteoimmunosenescence should significantly facilitate the development of therapeutic approach to correct this pathological process. It could be observed that the inflammation and senescence share multiple mechanisms, including ROS accumulation, shifted energy metabolism, NF-κB activation and inflammasome release, etc., however, it is still largely unknown how immune cell stay in the senescence status instead of cell death/apoptosis resulted from non-senescent inflammatory response. The mechanisms underlying immune cell senescence, especially the cause and division between inflammation and senescence should be further explored.

Based on the current knowledge, to improve the functional bone regeneration in the aged population, ideally, a biomaterial should be designed to target the osteoimmunosenescence by correcting the senescent immune microenvironment, therefore ensuring a favorable condition for the sequential osteogenesis. Such a biomaterial can be designed following the three principles suggested here: firstly, the physiochemical properties of material should be controlled to favor inflammation-inhibition (and to avoid triggering inflammation); secondly, the immunomodulatory property is indispensable for material design, which shall combine both inflammation-control and senescence-targeting strategies to resolve inflammaging; lastly, after correcting the inflammaging environment, osteoinduction factors can be responsively released to facilitate bone regeneration.

To achieve these three principles, a “smart” drug delivery system is required to ensure a targeted modulation of senescent immune cells, and to responsively release osteoinductive factors after inflammaging resolution (Figure 8). This can be achieved by nanoparticles with dual-responsive sequential release of different drugs, which is supposed to firstly release immunomodulatory factors to responsively regulate inflammaging, and then release osteoinductive factor in response to microenvironmental change. Considering the shared features such as ROS and pH between senescence and inflammation environment, ROS and pH can be utilized to trigger the sequential drug release (as discussed in Section 4.1.1).

For the selection of immunomodulatory factors, previously reported inflammation-inhibitory molecules (discussed in Section 4.1.1) can be used; moreover, considering the abnormal features of senescent immune cells (e.g., low plasticity and phagocytosis capacities), senescence-targeting strategies should be considered. As the major contributors in senescence (discussed in Section 3.1), ROS are considered as critical target for inflammaging control, and ROS-scavenging approaches, including the delivery of natural enzymes, small molecular drugs and more importantly, ROS-scavenging nanozymes could be used to correct inflammaging. In the future, nanomaterials deliver ROS scavenging biomolecules or ROS-scavenging nanozymes could be considered as efficient tools, which are expected to correct osteoimmunosenescence by inhibiting cellular senescence and reducing inflammaging, thus regulating the aging inflammatory environment and improving the functions of both macrophages and MSCs. In addition, the senolytic drugs (summarized in Section 5) to facilitate senescent cell clearance can also be considered, which can be delivered by advanced nanosystems to extinguish the senescent macrophages and/or senescent MSCs, thus relieving the inflammaging environment to benefit aging bone healing. Other senescence associated mechanisms (as mentioned in Section 3.1), such as mitochondria dysfunction, nonfunctional lysosomes/proteasome system, energy metabolism alterations, declined NAD^+ level, and reduced autophagy, can be considered as potential targets to design biomaterials for tackling cellular senescence. After inflammaging-control, the sequential release of osteoinductive factors (e.g., BMPs) can either utilize the same stimulant or not (e.g., ROS-responsive release of first inflammaging-control factors and then BMP2, or ROS-responsive release of first inflammaging-control factors and then pH-responsive release of BMP2), whereas to ensure a sequential responsive release to a single stimulation, the two delivery system should be able to release factors according to different levels of the stimulant (e.g., the inflammaging-control factors should be released in response to high ROS level, while BMP2 should be released in response to low ROS level).

In clinical, the application of nanoparticle in bone defect treatment requires a carrier to retain them at the defect site. A simple way to achieve this is to either use hydrogel or collagen as the base material to carry the nanoparticles, or to use nanoparticle as surface coating/surface deposition on bone implants. The drawbacks are obvious, as the former one is unable to provide mechanical support, and the later one is uncapable of inducing a functional bone regeneration that is, the implant should allow for new bone ingrowth during its degradation, and facilitate the replacement of implant with host new bone tissue. To achieve this goal, ideally, a porous implant is preferred to allow for the infiltration of host cells and ingrowth of blood vessel, nerve, and new bone. Therefore, a feasible design (Figure 8) is to use the nanoparticles as a proportion of raw material for 3D printing, an efficient approach to fabricate a personalized scaffold implant with predesigned structure. Considering both the properties of nanoparticle (summarized in Section 4.1.2) and implant (summarized in Section 4.2) can influence the local immune response, these properties, especially the raw material constitute, the shape/size of nanoparticle, the surface properties of bone implant, and the inner porosity of implant should be well-designed. Such a complicated design can be aided by computer-based analysis/modelling (e.g., CFD, density-functional theory (DFT) calculation, etc.) and machine learning approaches in the future. In addition, to apply the osteoimmunosenescence modulatory materials in aging bone healing, the long-term efficacy, safety (especially for nanomaterials, their potential side-effects such as liver accumulation/toxicity must be considered), and scalability should be considered and examined in material design and development. The ideal osteoimmunosenescence modulatory materials, which combining the advantages of implants (for structural support) and drug-delivery nanoplatforms, are designed with immunomodulatory physiochemical properties at both implant- and nano-level, are expected to significantly improve bone healing in aged patients owing to their unique capabilities to correct the inflammaging environment and restore senescent cell function, and to induce osteogenesis at the ideal condition (after inflammaging correction).

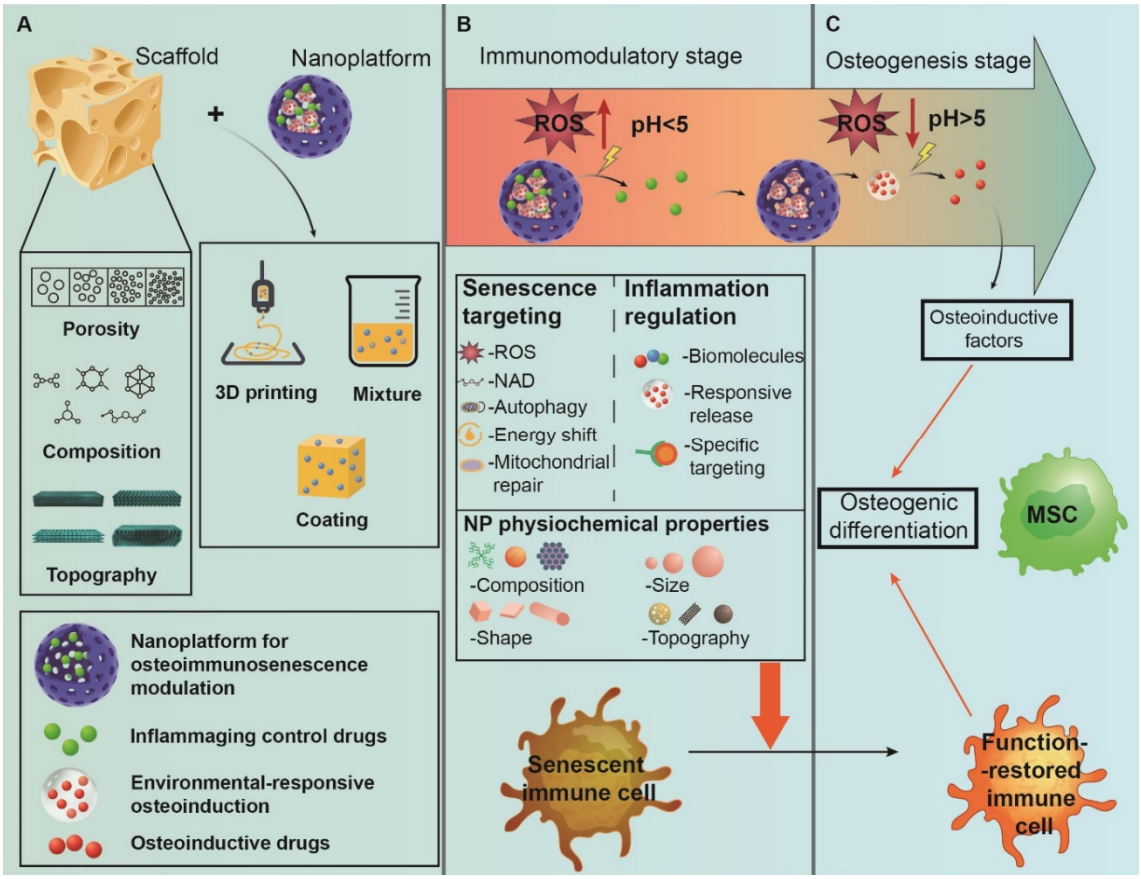

Figure 8. Schematic illustration of the proposed design for osteoimmunosenescence modulatory biomaterials. (**A**) Osteoimmunosenescence modulation can be achieved by combining the implant scaffold (properties should be considered) and advanced drug-delivery nanoplatforms; (**B**) The nanoplatform shall enable a dual delivery of inflammaging regulators to be released responsively to inflammatory environment, and generate an ideal immune environment for osteogenesis; and (**C**) osteoinductive factors to be released in response to environment correction and induce osteogenic differentiation of MSCs.

7. Conclusions

Taken together, in this review, we summarized the current finding in aging-associated abnormities in immune system and bone healing. Especially, the senescent immune-skeletal cell interaction, termed osteoimmunosenescence in this review, is considered as the major obstacle for bone regeneration in the aged population. Osteoimmunosenescence is characterized as inflammation, accumulation of senescent cells, and retarded osteogenic differentiation, in which the senescence-resulted decline in immune cell phagocytosis, and paracrine senescence (to covert young cells into senescent status) are the major reason for senescent cell accumulation, which produce SASP to contribute the inflammaging environment and further exacerbating both phagocytosis decline and paracrine senescence. The reason for senescence resulted SASP production could be attributed to mitochondrial dysfunction, ROS accumulation, energy metabolism change, NAD^+ decline and reduced removal of damaged mitochondria (deficiency in mitophagy) and self-garbage (deficiency in autophagy). All these contribute to two vicious circles to exacerbate inflammaging, one is the intracellular mitochondria damage-ROS-mitochondria damage circle, the other is the intercellular senescent cell accumulation-inflammation-senescent cell accumulation circle. Therefore, two inflammaging-resolution strategies are proposed; one is inflammation control, the other is to target senescent cells; moreover, the current material/pharmaceutical approaches for the two above-mentioned strategies are summarized. Furthermore, future directions on osteoimmunosenescence and materials science are proposed, and a potential guideline is provided to develop the osteoimmunosenescence-targeting biomaterials to improve bone regeneration. This review will therefore pioneer the development of bone regenerative materials for the aged population. By advancing our understanding of osteoimmunosenescence and developing targeted biomaterials, we have the potential to revolutionize bone regeneration therapies for the aging population and significantly improve patient outcomes.

Author Contributions

L.X.: conceptualization, writing—original draft preparation; W.G.: visualization, writing—original draft preparation; J.W.: writing—original draft preparation; I.E.: writing—original draft preparation; A.D.-P.: writing—original draft preparation; J.S.-C: writing—original draft preparation; Y.Z: writing—original draft preparation; A.R.S: writing—original draft preparation; I.P.: writing—reviewing and editing; R.C.: writing—reviewing and editing, J.M.O: writing—reviewing and editing; G.O.: writing—reviewing and editing; C.W.: writing—reviewing and editing; Y.X.: conceptualization, supervision, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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