ADVANCED HEALTHCARE MATERIALS

REVIEW

Osteoimmunomodulation

Current Advances in Immunomodulatory Biomaterials for Bone Regeneration

*Jinkyu Lee, Hayeon Byun, Sajeesh Kumar Madhurakkat Perikamana, Sangmin Lee, and Heungsoo Shin**

Biomaterials with suitable surface modification strategies are contributing significantly to the rapid development of the field of bone tissue engineering. Despite these encouraging results, utilization of biomaterials is poorly translated to human clinical trials potentially due to lack of knowledge about the interaction between biomaterials and the body defense mechanism, the "immune system". The highly complex immune system involves the coordinated action of many immune cells that can produce various inflammatory and anti-inflammatory cytokines. Besides, bone fracture healing initiates with acute inflammation and may later transform to a regenerative or degenerative phase mainly due to the cross-talk between immune cells and other cells in the bone regeneration process. Among various immune cells, macrophages possess a significant role in the immune defense, where their polarization state plays a key role in the wound healing process. Growing evidence shows that the macrophage polarization state is highly sensitive to the biomaterial's physiochemical properties, and advances in biomaterial research now allow well controlled surface properties. This review provides an overview of biomaterial-mediated modulation of the immune response for regulating key bone regeneration events, such as osteogenesis, osteoclastogenesis, and inflammation, and it discusses how these strategies can be utilized for future bone tissue engineering applications.

1. Introduction

Bone tissue possesses the ability to self-heal; however, critical size defects can complicate bone formation and may lead to fracture nonunion. Bone grafting using autografts and allografts still remains the gold standard treatment method in clinical situations. However, such strategies have many shortcomings such as risk of infection, graft rejection, donor site morbidity, and limited availability of suitable grafts.[1] Biomaterial-based tissue engineering approaches have been proposed as an alternative therapeutic tool.[2] However, they often exhibit low therapeutic efficacies due to poor osseointegration of biomaterials tion. Endothelial cells are implicated in the vascularization of the bone and inability to evoke the complex endogenous tissue healing process. Although the main bone healing mechanism during the reunion of large bone defects is ossification, which is controlled by a number of inductive factors, recruitment of inflammatory cells involve regulation of the activity of osteoprogenitor cells and later stage bone remodeling is imperative.[3] Therefore, collective approaches to modulate a variety of signaling processes within bone healing environment should be adapted for regeneration of biologically functional bone tissue.

During complex bone fracture healing, the presence and function of different cell types such as osteoblasts, osteoclasts, endothelial cells, and mesenchymal cells are spatiotemporally regulated. For example, osteoblasts are the predominant bone-forming cells, which generally originate from mesenchymal cells while osteoclasts (i.e., multinucleated cells that are derived from hematopoietic progenitors in the bone marrow) activate bone resorp-

tissue. Although individual cell type independently plays important roles during bone tissue healing, strong paracrine effects from the immune system have been known to predominantly influence all these cellular activities.[4] This strong cross-talk between the skeletal system and immune system has led to a new direction in the research, referred to as osteoimmunology. For example, cytokines produced by immune cells such as transforming growth factor (TGF)-β and interleukin (IL)-4 have been demonstrated to induce osteoblast migration, proliferation, or secretion of bone extracellular matrix (ECM) during the early stage of differentiation.[5] In contrast, IL-1β and tumor necrosis factor (TNF)-α have been implicated in the inhibition of osteoblast differentiation.[6] Proinflammatory cytokines produced by immune cells such as TNF-α and IL-1 induce the osteoclast cell activity, with in vivo studies suggesting that blocking TNF-α and IL-1 showed reduced bone loss in postmenopausal osteoporosis conditions.[5b,7]

In the last couple of decades, various biomaterial-mediated bone tissue engineering approaches have been attempted to modulate the activity of various cell types involved in bone regeneration to induce bone formation.[8] Majority of previous

J. Lee, H. Byun, Dr. S. K. Madhurakkat Perikamana, S. Lee, Prof. H. Shin
Department of Bioengineering
Hanyang University
222 Wangsimni-ro, Seongdong-gu, Seoul 04763, Republic of Korea
E-mail: hshin@hanyang.ac.kr
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adhm.201801106.

DOI: 10.1002/adhm.201801106

works involved direct activation of osteoprogentors responsible for osteogenesis. However, growing evidence suggests that the physiochemical properties of implanted materials can also evoke an inflammatory response at the injury site, which can positively or negatively affect bone regeneration. For example, screws made of magnesium alloy ZEK 100, a well-known orthotropic implant material, showed higher inflammatory response and bone volume loss when implanted into a rabbit tibia model.[9] Hence, more attention should be paid to biomaterial design to modulate their cross-talk with the immune system and avoid undesirable inflammatory responses and to modulate the activity of bone-forming cells.

In this review, we initially provide a general overview of the inflammatory process and the immune system. We also highlight the specific roles of macrophages in the inflammation and wound healing process. The following section briefly addresses the biomaterial–immune system cross-talk and how macrophage polarization can be controlled by the specific biomaterial properties. In the later sections, we provide a detailed discussion about how macrophage modulation by biomaterials can be used to control various events in bone tissue engineering such as osteogenesis, osteoclastogenesis, and inflammation.

2. Immune Responses and Immunomodulation by Biomaterials

2.1. Inflammation and Immune Responses

Inflammatory response (inflammation) occurs when tissues are injured by bacteria, trauma, toxins, heat, or any other causes. Generally, they can be divided into noninfectious damage-associated molecular patterns (DAMPs) and infectious pathogen-associated molecular patterns (PAMPs).[10] DAMPs are endogenous molecules that are constitutively expressed and released upon tissue damage, leading to activation of the immune system.[11] These molecules consist of intracellular proteins such as heat shock proteins, high-mobility group box 1 (HMGB1), inflammatory cytokines such as IL-1α, and small fragments of ECM during tissue damage.[12] PAMPs represent molecules associated with groups of pathogens and bacterial lipopolysaccharides (LPSs); endotoxins found on the cell membranes of Gram-negative bacteria are considered to be the general class of PAMPs.[11] These danger signals (including DAMPs and PAMPs) activate the immune system, which includes a complex network of cells, tissues, and organs that work together to defend against inflammation. In general, these processes involve various pattern recognition receptors such as the toll-like receptors (TLRs) and receptors for the advanced glycation end products (RAGE), which are present on the antigen presenting cells (such as dendritic cells and macrophages). These receptors induce a response through the activation of transcriptional factors such as nuclear factor kappa-light-chainenhancer of activated B cells (NF-kB) or interferon regulatory factors.[13]

Immune cells play a pivotal role in regulating the immune system against inflammations. These cells mainly include neutrophils, mast cells, dendritic cells, T cells, monocytes, and macrophages.[14] Neutrophils are usually recruited soon after the injury and are responsible for wound detection and removing the contaminants.[15] They secrete a number of antimicrobial substances and proteases that can hinder the pathogen growth at the wound sites. Besides, neutrophils secrete various cytokines and growth factors, which can further trigger the migration of more neutrophils and other immune cells such as macrophages to the injury sites.[16] In fact, a recent report suggests that neutrophils regulate the macrophage recruitment and polarization, and improve the cardiac repair in a mouse acute myocardial infarction model.[17] Dendritic cells are antigen presenting cells involved in the phagocytosis of antigen materials and the tissue healing process.[18] T cells possess many subtypes, and there has been much evidence to show the potential involvement of T cells in cytokine production and tissue repair, although the exact functions and mechanisms of different subtypes have yet to be well elucidated.[19] Although multiple immune cell types are involved in the immune response, macrophages play the most important role in tissue remodeling by secreting various cytokines, and they are also responsible for phagocytosis of unwanted materials and recruitment of other cells to the injury site.[20] Therefore, the role of macrophages has been investigated vigorously in various tissue regeneration processes, and that information could inform the regenerative medicine approaches. Macrophages can originate either from tissue resident macrophage populations or by the recruitment and differentiation of circulating monocytes from the blood during tissue injury (the latter being considered as the predominant source of macrophage during tissue healing).[21] Circulating monocytes usually invade the injury site and differentiate into macrophages during the initial time with their peak accumulation period being 4–7 days after injury.[22]

Macrophages possess high plasticity (polarization), easily switching their functional phenotype to another in response to the stimuli received from local changes in their microenvironments. Classically, macrophages are polarized into two functionally and phenotypically distinguishable subtypes of 1) the proinflammatory "M1" and 2) the anti-inflammatory "M2" phenotypes.[23] Those arriving at the injury site at the initial stage often possess M1 phenotypes, and thus are involved in the phagocytosis of dead cells and pathogens. In addition, they produce a number of proinflammatory cytokines such as TNF-α, iNOS, and IL-12.[24] Various in vitro characterization data provided clues for macrophage polarization, in which interferon (IFN)-γ, LPS, or TNF-α and M-CSF serve as key stimulators for M1 polarization of macrophages. The M1 phenotype typically stays at the injury site 3–4 days after injury, and thereafter, the phenotype changes to M2. The persistence of M1 phenotype for a prolonged time period and failure to enhance the M2 phenotype could lead to the inability of macrophages to resolve the inflammation, eventually resulting in poor tissue healing. Typically, they express crucial genes in wound

Heungsoo Shin is currently a professor in the Department of Bioengineering, Hanyang University, Republic of Korea. His main research thrusts in his laboratory include the development of biomimetic materials for regenerative medicine, the development of carriers for cell and growth factor delivery, and systemic characterization of cell–extracellular matrix interactions. His works particularly have led to innovative approaches in regeneration of impaired bone, muscular, and vascular tissue.

repair (such as arginase and Fizz1) and secrete various cytokines and growth factors that help in cell proliferation, differentiation and ECM depositions including IL-4, IL-10, and TGF-β.[25] They also possess a very strong paracrine signaling process between the phenotypes. For instance, secretion of inflammatory cytokines such as TNF-α and IL-12 produced by the M1 phenotype can be regulated by an M2 polarizing cytokine such as IL-4 and IL-10 produced by the M2 macrophages, which are residing in close proximity in the microenvironment.[26] Hence, a spatiotemporal activation of macrophages and their precise control on M1– M2 polarization throughout the tissue repair process can be a promising strategy in therapeutics, and such approaches have not been well explored in the tissue engineering community.

2.2. Immunomodulation by Biomaterials in Tissue Engineering

Biomaterials have been widely used in tissue engineering since they can provide structural support to the damaged tissue and serve as a carrier for delivery of molecules such as drugs, growth factors, genes, and cells.[27] Biomaterials can be broadly classified as natural polymers such as collagen, elastin, alginate, and hyaluronic acid or synthetic polymers such as poly(ι-lactic acid) (PLLA) and polycapolactone (PCL).[28] It has been widely accepted that cell response can be affected by the microenvironmental cues from the biomaterials. For example, biophysical properties such as material stiffness, topography, and geometry of pores can influence the cellular response. When neuronal stem/progenitor cells were cultured on a methacrylamide chitosan substrate with varying stiffness, their neuronal differentiation was favored on the softest substrate (<1 kPa) rather than the rigid substrates.[29] Similarly, anisotropic morphology of the biomaterial has been highly favored for stem cell migration, endothelial cell adhesion and maturation, and myoblast differentiation.[30] Biochemical properties such as surface chemistry, ligand density, the presence of biomolecules, and degradation rate of the materials may influence the cell adhesion, proliferation, differentiation, and ultimately tissue regeneration.[31] For instance, various surface functional groups have been introduced on a polyethylene glycol (PEG) hydrogel (including amino, *t*-butyl, phosphate, fluoro, and acid groups), demonstrating that osteogenic differentiation of human mesenchymal stem cells (hMSCs) was favored by hydrogels with phosphate functional groups. Furthermore, their chondrogenic differentiation was enhanced on acid-functionalized hydrogels, whereas hydrogels with *t*-butyl groups maximized the adipogenic differentiation of hMSCs.[32] Hence, present bone tissue engineering approaches have focused on engineering biomaterial surface that can modulate various events during the bone tissue regeneration such as immune response, osteogenesis/ osteoclastogenesis, and infection or inflammation and such approaches can orchestrate the bone healing and implant integration to the host body (**Figure 1**).

In general, biomaterials implanted into the body result in a complex inflammatory response leading to a foreign body response.[36] These inflammatory responses trigger a variety of biochemical signals and induce the recruitment of different immune cells to the region of the implanted biomaterials. Recruited monocytes are then differentiated into macrophages in the presence of various proinflammatory cytokines produced by the other immune cells. These macrophages then adhere on the implanted materials, and the physicochemical properties of the materials play significant roles in macrophage plasticity, which influence the tissue healing process.[37] It has been reported that the immune response to biomaterials may be attributed to the interactions between various proteins and the implanted biomaterials. The types, concentration, and mode of protein adsorption may change depending on the biomaterial properties, and they can later also lead to conformational changes in the protein structure.[38] More notably, all these parameters can also regulate the activities of immune cells (**Figure 2**a).[39] The interactions between the adhesion receptors of immune cells and these adhered proteins are the main mediator of various signaling cascades. For example, adsorbed fibrinogen showed a proinflammatory response, while soluble fibrinogen showed no such response. Fibrinogen adsorption and denaturation on the biomaterial substrate exposed a short sequence in the fibrinogen D domain, namely, γ190-202, P1, and γ377-395, P2, which interacted with the integrin Mac-1 of immune cells and made them proinflammatory. Extended studies from their research also indicated that these inflammatory responses also depend on the biomaterial types as materials such as polyethylene terephtahalate (PET), and polyvinyl chloride (PVC) showed more P1 and P2 epitope exposure and inflammatory response than the other tested materials (poly(ether urethane) (PEU) and polydimethylsiloxane (PDMS)) in an in vivo mice experiment.[40] Implanted biomaterials have shown the ability to tune the macrophage response based on their intrinsic material properties, broadly classified into biological, chemical, and physical properties. Biomaterials derived from native ECM (such as hyaluronic acid) have shown excellent anti-inflammatory properties and prevented liver injury by inhibiting the production of proinflammatory cytokines in a T cell–mediated mice liver injury model.[41] Another in vivo mimicking biological polymer, sulfated alginate, promoted M2-like polarization of human THP-1 monocytes and decreased their proinflammatory cytokine production.[42]

Significant efforts have also been made to modify the synthetic biomaterial substrates with biological properties such as anti-inflammatory drugs or cytokines for delivering at injury site or in a surface coated form. For example, silk fibroin–functionalized electrospun PCL nanofibers have been decorated with anti-inflammatory cytokine IL-4 by a layer-by layer assembly, and the modified nanofiber promoted the M2 macrophage polarization in a murine subcutaneous model (Figure 2b).[33] Silicon neural probes coated with an anti-inflammatory drug, dexamethasone, reduced the inflammatory response in a rat

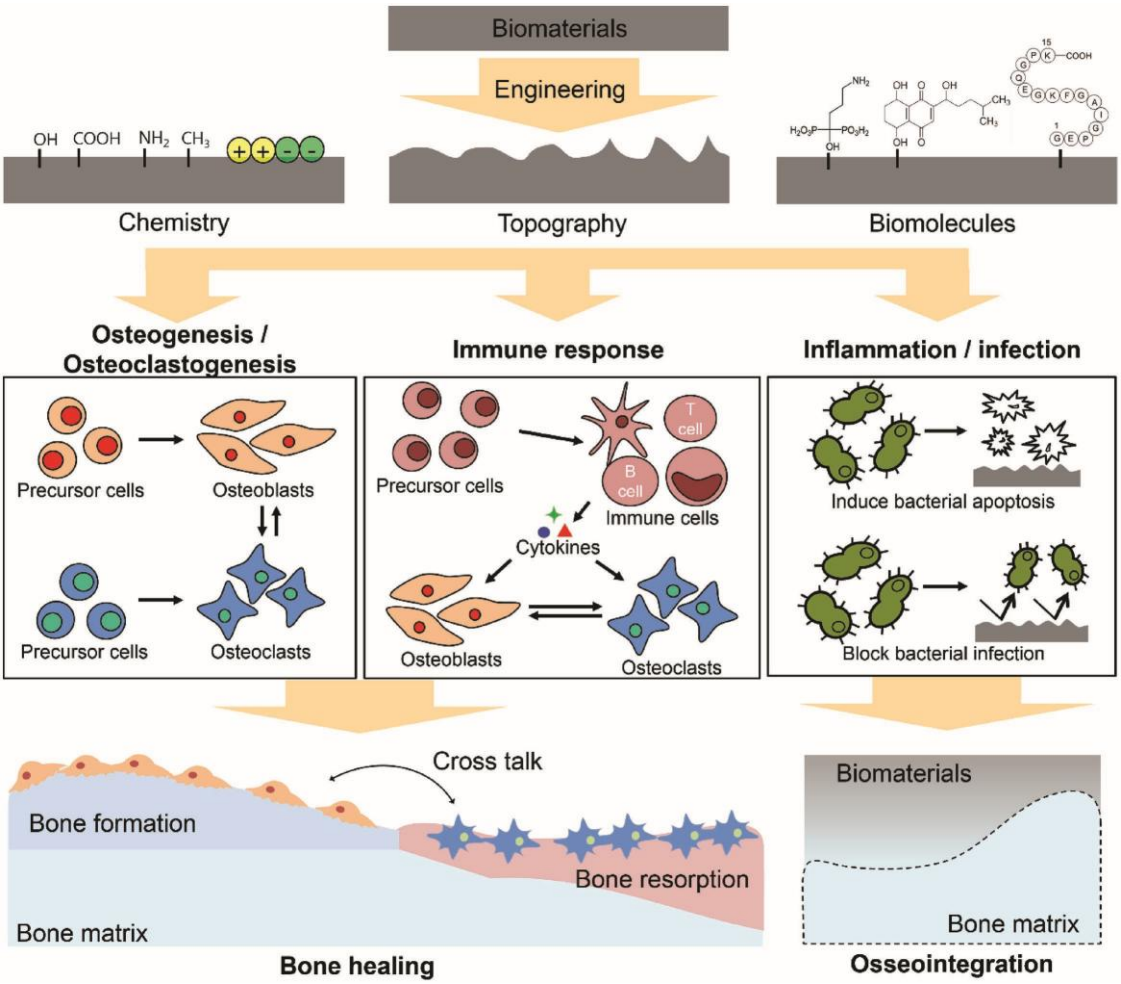

Figure 1. Biomaterial engineering for modulation of various events during bone healing associated with inflammation, infection, immune response, osteoblastogenesis, and osteoclastogenesis.

brain model and improved the astroglial response with the prevention of the neuronal loss.[43] Use of nano-biomaterials for the controlled delivery of various biomolecules for controlling macrophage polarization has been increasingly finding the interest in recent years.[44] For example, mesoporous nanoparticles with a porosity of 30 nm have shown excellent loading capacity of IL-4 and their injection into an in vivo mouse model resulted in greater M2 polarization.[44a] In another interesting attempt from the Liming Bian group, remote manipulation of macrophage responses was achieved by conjugating RGD-grafted gold nanoparticles to a glass substrate and subsequently a magnetic nanocage was prepared on the nanoparticles through a flexible linker, which allowed a magnetic manipulation of reversible caging and uncaging of the RGD bearing nanoparticles. Results from their further experiments showed that uncaging of RGD temporarily promoted M2 polarization of macrophage and inhibited M2 polarization both in vitro and in vivo.[44b] Similarly, the physical properties of the biomaterials (such as stiffness, topography, and porosity) have shown a strong impact on macrophage polarization. For example, studies conducted by McWhorter et al. showed that using substrates with a micropatterned macrophage cell shape can be modulated, and macrophages with elongated shapes showed more M2 polarization (Figure 2c).[34] Mouse bone marrow–derived macrophages were cultured on polydioxanone electrospun fibers with varying pore sizes, demonstrating that increased pore size favored the M2 phenotype of the macrophages, which was evident by the increased expression of Arginase 1 and decreased expression of M1 marker iNOS.[45] Similarly, human macrophages cultured on collagen-based matrices with varying stiffness showed that increased matrices stiffness resulted in an M2 phenotype of the macrophages.[46]

Modification of biomaterial surface chemistry is another widely employed strategy that can directly modulate the macrophage response. The surface chemistry of the material can influence the protein adsorption and further downstream signaling processes with the immune cells. Various surface chemical features such as the type of functional groups, surface charge, change in hydrophilicity, and molecular weight of the compound have a critical influence on the immune response in a mouse model. An extensive study was conducted by Bygd et al. in which they screened a library of chemical functional groups based on their influence on macrophage polarization between M1 and M2 phenotypes by using

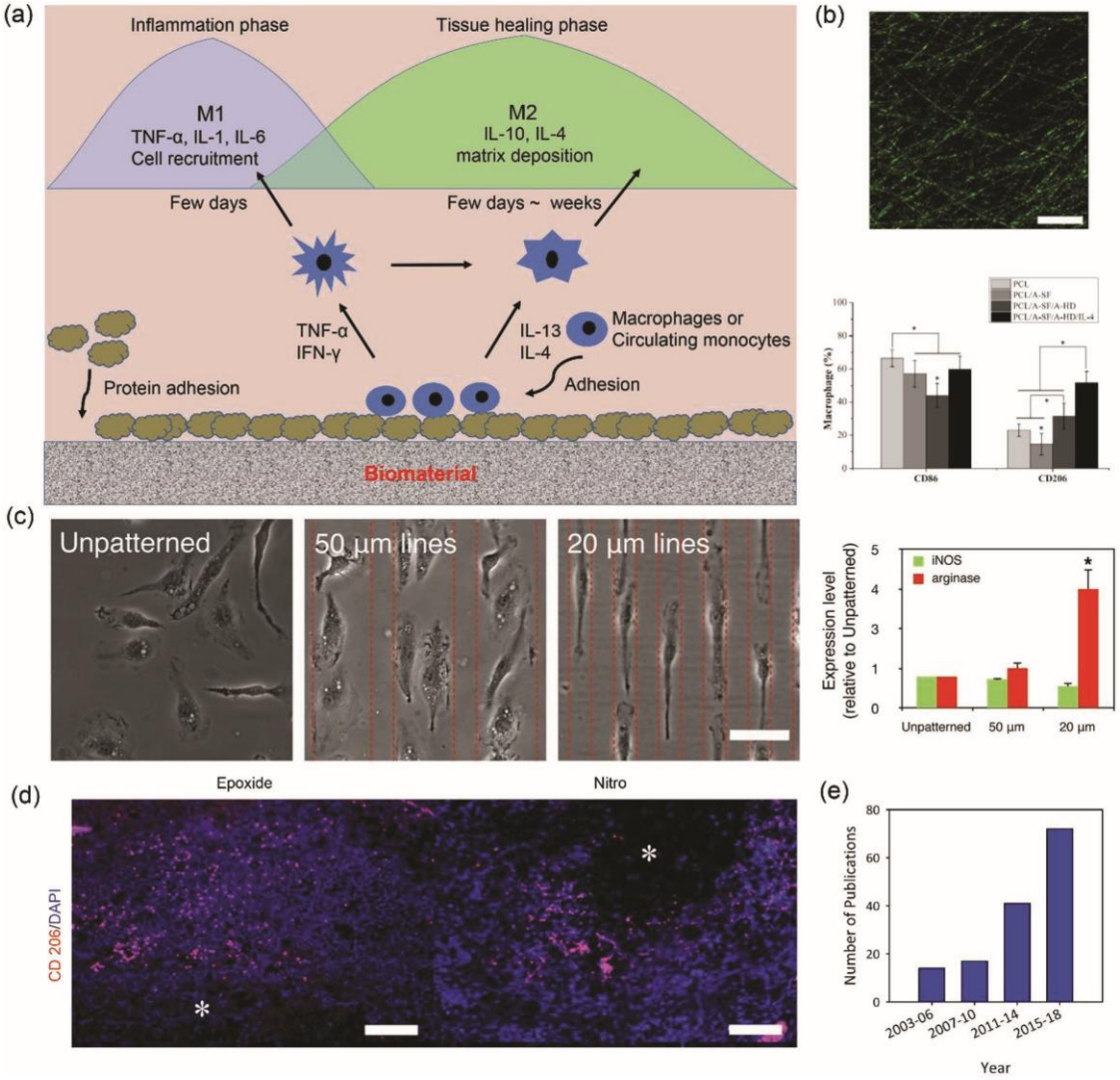

Figure 2. a) General schematic of the biomaterial modulation of macrophage response in tissue healing. b) Immunofluorescence image showing specific adsorption of IL-4 on a silk fibroin–functionalized PCL nanofiber and IL-4 incorporated scaffold showing more M2 phenotypic macrophage adhesion when implanted in a subcutaneous model. Reproduced with permission.[33] Copyright 2018, Elsevier. c) Phase contrast images of bone marrow–derived macrophages on unpatterned and patterned PDMS substrates, and a relative protein expression level of different macrophage polarization markers including iNOS(M1) and arginase (M2). Reproduced with permission.[34] Copyright 2013, United States National Academy of Sciences. d) Biomaterials functionalized with different chemical functional groups showing different level of CD 206 positive cells in an in vivo mouse model. Reproduced with permission.[35] Copyright 2015, Elsevier. e) The number of yearly publications on immunomodulation for bone tissue engineering was estimated through a PubMed search using the key word "immunomodulation for bone tissue engineering" on September 18, 2018.

poly(*N*-isopropylacrylamide-*co*-acrylic acid) nanoparticles modified with a variety of functional groups including phosphonic acid, alkene, epoxide, amide, ether, and nitro or oxime groups (Figure 2d).[35] In another study, a carbon nanowall surface was plasma polymerized with carbon, nitrogen, and oxygen functional groups, and the presence of oxygen functional groups increased the macrophage adhesion and activation.[47] Similarly, polyethylene terephthalate films with hydrophilic/neutral surfaces inhibited macrophage adhesion and fusion; however, adherent macrophages on these substrates produced significantly higher amounts of various cytokines and chemokines than their hydrophobic and hydrophilic/ionic counterparts.[48] Bartneck et al. also demonstrated that the surface charge of the material can be used to modulate the polarization state of the macrophages.[49]

The above examples show that changes in the biomaterial properties can strongly influence the macrophage polarization, and by extension, the wound healing characteristics.

In the last few decades, there has been a great advance in various interdisciplinary fields associated with the biomaterial research such as material chemistry, material fabrication techniques, and nanotechnology. All these have significantly contributed to the application of biomaterials in the field of tissue engineering. However, the tissue engineering community has not until recently given much focus on the biomaterial–immune system interactions, and there is a lack of understanding about how pathological changes due to the biomaterials can alter the wound healing process. Hence,

a significant portion of the current biomaterial research has now started to explore various surface modification strategies to fine tune the immune system, either to eliminate the unwanted inflammation or to reprogram the immune cells to contribute to the tissue healing process. In particular, bone tissue healing is strongly influenced by the cross-talk between immune cells and various bone-forming cells such as osteoblasts, stem cells, and osteoclasts. Therefore, it is important to understand how the biomaterial–immune cell interactions can contribute to bone regeneration. Such studies are recently finding interest among bone tissue engineering community as the number of yearly publications on immunomodulation for bone tissue engineering significantly increased, which was estimated through a PubMed search using the key word "immunomodulation for bone tissue engineering" on September 18, 2018 (Figure 2e).

3. Biomaterials for Modulation of Osteogenesis

3.1. Immune Responses and Osteogenesis

Osteogenesis is the major process of bone forming in which early bone is developed and ECM is mineralized.[50] Osteoblasts play an important role in this process by mineralizing osteoid, which is ECM of bone tissue. Osteoblasts originate from MSCs and precursors of various mesenchymal lineage cells such as chondrocytes, osteoblasts, adipocytes, or myoblasts.[50b] Differentiation of MSCs into osteoblasts is called osteoblastogenesis. Once MSCs are differentiated into osteochondrocyte precursors, several molecules (including Runx2, β-catenin, or Dlx3/5/6) induce precursors that become immature osteoblasts. Then, maturation of immature osteoblasts is conducted by many proteins including osterix, NFAT, and β-catenin. In addition, these factors activate osteocalcin, osteopontin, and osteonectin for final differentiation of mature osteoblasts into osteocytes or lining cells.[50b,51]

Immune responses are closely related to the generation of new bone tissue. In the initial stage of bone regeneration, acute inflammation occurs and initiates bone tissue regeneration in response to external factors such as infection. The initial inflammation induces recruitment of stem cells and immune cells such as T-cells or monocytes.[50a] Recruited immune cells can produce cytokines such as TNF and IL-6, which are important for differentiation of mesenchymal stem cells into osteoblasts.[50a] However, infection may cause prolonged inflammation, and the remaining proinflammatory cytokines negatively affect bone regeneration.[52] Systemic circulation of these cytokines including IL-6, TNF, and IL-1 induces a humoral immune response both at the injury site and in the whole body. These persistent events result in several degenerative diseases including osteoarthritis, rheumatoid arthritis, and osteoporosis.[52] From this perspective, the regulation of inflammation in the initial stage of bone regeneration is a promising area in biomaterial-based tissue engineering.[50a,52]

Like other inflammatory responses during wound healing, macrophages play important roles in bone regeneration. M1 and M2 type macrophages highly affect the microenvironment of bone regeneration site via cytokine secretion. The cytokines secreted by macrophages both regulate inflammation and control the differentiation of MSCs and the function of osteoblasts. M1 macrophages secrete inflammatory cytokines including TNF, IL-1β, IL-6, IL-12, and IL-23.[52,56] INF-γ, which is an inducible factor for M1 macrophages and IL-1, interferes with osteoblasts resulting in the synthesis of collagen. IL-1β and TNF-α also suppress the synthesis of alkaline phosphate by osteoblasts and negatively affect secretion and mineralization of extracellular bone matrix.[57] M2 macrophages release anti-inflammatory cytokines including IL-10, TGF-β, and IL-1RA.[52,56] IL-10 is known to enhance differentiation of osteoblasts as shown in a study with IL-10 depleted mice.[58] TGF-β is upstream of bone morphogenetic protein (BMP) signaling, which is a well-known osteogenic factor and finally induces osteoblast differentiation of MSCs.[59] IL-1RA is an inhibitor of IL-1, which is one of the main proinflammatory cytokines that consequently promotes osteoblasts by regulating the adverse effects of IL-1. IL-4, an inducible factor of M2 macrophages, also affects osteoblasts by inhibiting differentiation, resulting in reduced bone mass. However, it can also accelerate proliferation of osteoblasts.[52] In this view, modulation of immune response is now highlighted for its role in both the inflammation and formation of bone (**Figure 3**a).

3.2. Immunomodulation by Biomaterials for Controlling Osteogenesis

A number of studies have been performed to control the function of osteoblasts to modulate bone regeneration as shown in **Table 1**. Employing biological molecules or minerals as stimulatory engineered microenvironments for osteoblasts has been widely used for this purpose.[51b] Although a majority of previous approaches have been focused on direct control of osteoblasts, there have also been many reports on the use of biomaterials to enhance osteogenesis via immunomodulation. [60a,62a,68] Since cytokines have complex properties affecting the control of inflammation as well as osteogenesis, these studies have been focused on controlling macrophages or delivery of cytokines to regulate osteoblast function. In addition, many of these studies demonstrated the use of a combination of various factors that can simultaneously affect overall bone regeneration such as osteoclasts or mesenchymal stem cells.[62a,69] Therefore, it is important to choose an appropriate approach to reach the intended goal for bone regeneration.

3.2.1. Chemical Properties of Biomaterials for Osteogenesis

Surface modification of biomaterials has been widely used to present a variety of surface properties by employing hydrophilic functional groups, surface charge, and biominerals. Hydrophilicity is one of the major chemical properties required for biomaterials due to their accessibility to biological molecules via functional groups. There are several methods to make the surfaces of biomaterials hydrophilic such as plasma modification or hydrolysis.[70] Surface charges related to the hydrophilicity are also used for surface modification of biomaterials. Adsorption of biological molecules, which has a significant influence on osteogenesis, is highly affected by surface charge.[71] Previous

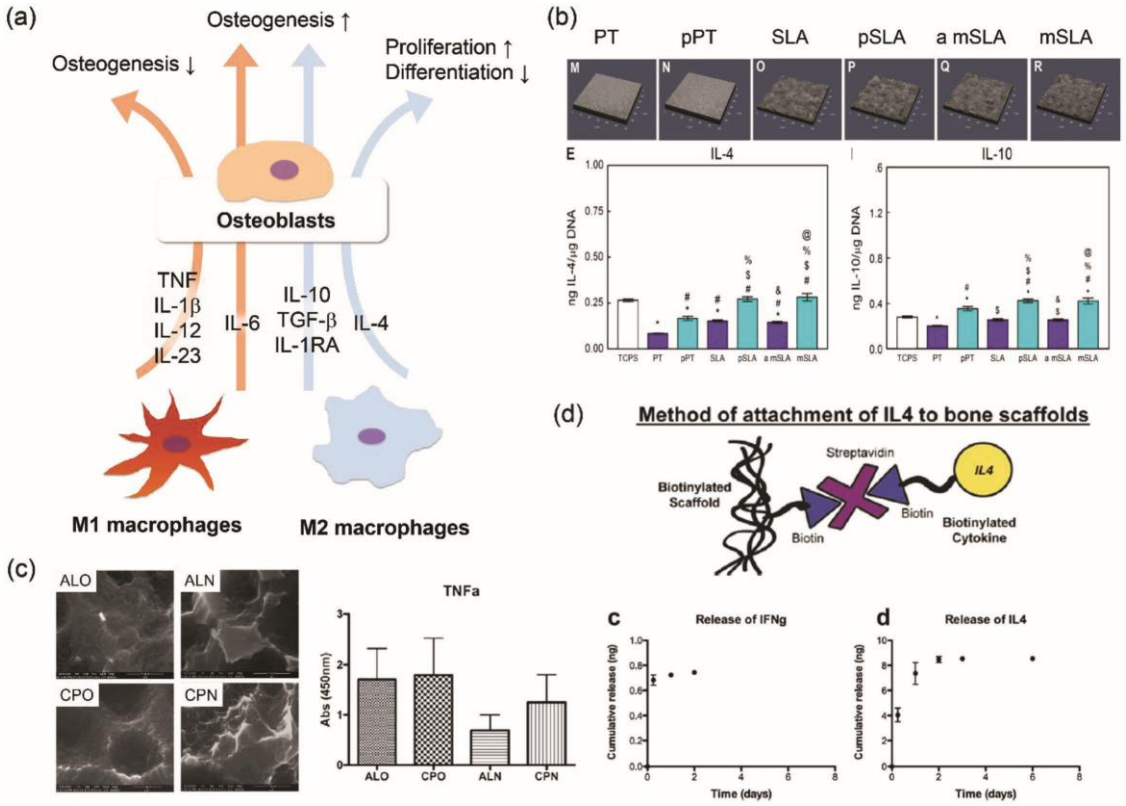

Figure 3. a) Schematic illustration of the effects of various cytokines secreted from macrophages on osteogenesis. b) SEM images of Ti surface prepared from various fabrication methods (PT: pretreatment; pPT: plasma-treated PT; SLA: sandblast and acid etching; pSLA: plasma-treated SLA; a mSLA: aged mSLA; mSLA: SLA modified with hydrophilic groups) and secretion of IL-4 measured by ELISA. Reproduced with permission.[53] Copyright 2016, Elsevier. c) SEM images of mineralized Ti surface (ALO: alloy with microroughness; CPO: commercially pure Ti with microroughness; ALN: alloy nanoscale CaP-modified; CPN: commercially pure Ti nanoscale CaP-modified) and their effects on TNF-α secretion of macrophages. Reproduced with permission.[54] Copyright 2011, Elsevier. d) Schematic illustration of the functionalization of cytokines via a biotinylation process (top) and release profile the loaded cytokines (IFNγ: mechanically loaded; IL-4: chemically functionalized). Reproduced with permission.[55] Copyright 2015, Elsevier.

Table 1. Biomaterial strategies for modulation of cross-talk between immune response and osteogenesis.

Engineering parameters	Property		Regulatory effect on immune response		Effect on osteogenesis	Ref.
			Upregulation	Downregulation		
Surface chemistry	Hydrophilicity		TGF-β, BMP IL-10, TNF-α, IL-4	TNF-α, IL-1α, IL-1β, Ccl-2, IL-6	(+)	[53,60]
	Surface charge	Anionic	IL-10	IL-8	(+)	[61]
		Cationic		IL-10, IL-1RA	(−)	
	Biominerals		BMP-2, IL-1RA, TGF-β1	TNF-α, IL-1β, IL-6	(+)	[54,62]
Topography	Roughness		TGF-β, IL-4, IL-10, IL-6		(+)	[53,60a,c]
	Micropattern (alignment)		IL-10	TNF-α	(+)	[63]
	Large pore		Arginase	iNOS, IL-1R1	(+)	[45,64]
	High porosity		Arginase		(+)	[45]

Delivery of biomolecules	Cytokines	Oncostatin M	ALP, STAT-3		(+)	[55,65]
		IL-4	ALP, IL-1RA	TNF-α,		
		IL1RA		TNF-α, IL-1β, IL-6		
	Proteins	BMP-2		IL-1β, IL-6, iNOS	(+)	[66]
	Nucleic acids	Noncoding RNA MALAT1	IDO		(+)	[67]

works have usually used materials with either cations or anions for enhanced osteogenesis.[61,71] Additionally, the immobilization of minerals on bone implants plays an important role in controlling the function of osteoblasts. There have been a number of methods for biomineralization on the surface of biomaterials including thermal spraying, sputter coating, and the use of simulated body fluid.[72] Although various surface modification methods have been developed for bone regeneration, enhancement of osteogenesis via immunomodulation through the material surface chemistry has been reported recently.

Like other biomaterials used in tissue engineering applications, an increase in the hydrophilicity of materials has been reported to enhance osseointegration of an implant. Immune responses of materials influence the affinity of the cells for a material and the function of osteoblastic cells. Vlacic-Zischke et al. reported that surface-modified hydrophilic titanium (Ti) showed increased signaling related to cytokine production of osteoblasts compared to an unmodified hydrophobic surface. To make the hydrophilic surface, Ti disks were rinsed under N_2 and stored in an isotonic saline solution at pH 4–6. Osteoblasts were cultured on these substrates, and the hydrophilic Ti showed an increased level of TGF-β/BMP signaling. These results indicated enhanced cytokine receptor expression of osteoblasts on hydrophilic surfaces.[60a] The introduction of hydrophilicity to a material also affects the activity of macrophages. Hydrophilic Ti disks prepared under 10% H_2O_2 solution for 24 h increased the growth of murine RAW264.7 macrophages. Higher levels of IL-10 (an anti-inflammatory cytokine) and lower levels of TNF-α (proinflammatory factor) were measured in the hydrophilic Ti.[60b] Using the same type of macrophages, Hamlet et al. also demonstrated downregulation of the secretion of proinflammatory cytokine such as TNF-α, IL-1α, IL-1β and chemokine such as Ccl-2 from macrophages by culturing on hydrophilic Ti.[60c] In an additional study, Hotchkiss et al. used oxygen plasma to generate hydrophilicity on a Ti disk. The culture of the primary murine macrophages isolated from C57BL/6 mice onto the hydrophilic Ti showed downregulation of proinflammatory cytokines and upregulation of anti-inflammatory cytokines, indicating the polarization of macrophages into the M2 phenotype, which enhances the function of osteoblasts (Figure 3b).[53]

Surface charge plays an important role in the modulation of surface properties of materials. Brodbeck et al. used biomaterials based on acrylamide with anionic and cationic functional groups of poly(acrylic acid) and poly(dimethylaminopropylacrylamide), respectively. The anionic substrate promoted the secretion of IL-10 and decreased the secretion of IL-8. On the other hand, the cationic substrate inhibited production of IL-10 and IL-1RA, which are important for mature osteoblasts. These results indicated that anionic surfaces could promote osteoblast function, while a cationic substrate may promote secretion of proinflammatory cytokines and inhibit activation of osteoblasts.[61] On the other hand, divalent cations may polarize macrophages in different ways. In one study, Lee et al. used a Ti implant modified with Ca and Sr to culture J774.A1 macrophages. The macrophages cultured on the surface of Ti presenting Ca and Sr showed increased levels of markers for M2 macrophages, including arginase 1 and mannose receptors. These results suggested that a divalent cationic surface may enhance the function of osteoblasts in bone regeneration.[68b] Surfaces exhibiting biominerals have been widely used in the preparation artificial bone substitutes due to their potential to accelerate bone regeneration. Mineralized surfaces can induce osteogenic differentiation of stem cells, and thus enhance osseointegration. Meanwhile, osteoimmunology of minerals has also been an active area in bone tissue engineering. Hamlet and Ivanovski modified the surface of Ti with calcium phosphate and compared the reaction of macrophages with Ti with the same roughness. It was found that the nanocrystals of calcium phosphate resulted in downregulation of proinflammatory cytokines such as TNF-α and IL-1β, inhibitors of osteoblasts differentiation. These nanocrystals also increased adhesion of osteoblasts (Figure 3c).[54] Wang et al. also reported increased recruitment of MSCs, which can be a precursor to osteoblasts resulting in the secretion of osteoblasts that activate cytokines via culturing macrophages on biphasic calcium phosphate ceramics.[62c] Chen et al. used magnesium scaffolds with β-tricalcium phosphate (TCP), which accelerated macrophages to secrete BMP-2 and IL-1RA, which are inducible factors for osteoblasts.[62a] Wang et al. cultured macrophages on magnesium calcium phosphate cement made by sintering to observe the cross-talk between stem cells and immune cells. Macrophages seeded on the cement secreted more TGF-β1 and less TNF-α and IL-6, suggesting the potential use of biomineralized materials for enhancement of osteogenesis.[68a] Collectively, these studies show that mineralization of the materials appears to directly enhance osteogenesis as well as control immune cells to promote bone regeneration.

3.2.2. Physical Properties of Biomaterials for Osteogenesis

Besides the surface chemical properties, roughness, topography, porosity, and pore size of biomaterials can affect cellular function. In general, roughness may be present on a microscale, socalled microroughness, and microroughness on the surface of bone-biomaterials has been reported to enhance cell adhesion. Vlacic-Zischke et al. prepared Ti substrates with microroughness by using

a sand-blasted acid-etching method; they cultured osteoblasts on them and observed an increase in the level of TGF-β signaling and osteogenic potential.[60a] Hotchkiss et al. also prepared Ti surfaces with microroughness, demonstrating polarization of macrophages into an M2-like state with secretion of more IL-4 and IL-10 relative to a culture on a smoother surface. These cytokines are important for osteoblasts maturation (Figure 3b).[53] However, Hamlet et al. reported that secretion of proinflammatory IL-6 was also increased in macrophages cultured on Ti with microroughness.[60c] It seems that the microroughness of the biomaterial enhanced both M1 and M2 phenotype cytokines. Recently, nanoscaled roughness has been studied by exploiting advances in micro/nano fabrication technology. Kunzler et al. applied positively charged nanoparticles on PEI coated silicon wafers to control adhesion of osteoblasts, and decreased adhesion and proliferation on area with higher density of nanoparticles were found.[73] Furthermore, effects of nanoroughness on osteoimmunity were also investigated. For example, the different size of gold nanoparticles (16–68 nm) on allylamine surface balanced secretion of cytokines in nanoroughness groups to enhance osteogenesis.[74] Ma et al. modified surface of TiO_2 with UV irradiation to give different roughness (6–12 nm). They implanted these materials in rats and observed higher secretion of proinflammatory immune response in implant with higher roughness. Unlike random roughness, microfabrication techniques have been utilized to create patterns with intended size and shapes to biomaterial surfaces.[75] A number of cell types showed their ability to recognize specific shapes, and thus, a pattern-dependent cellular response was achieved. For example, elongated macrophages on cultured stripe patterns tended to be polarized into an M2 phenotype.[76] McWhorter et al. reported that the shape of macrophages can undergo direct polarization into M1 or M2. In this paper, elongation of cell shape lead to the expression of M2 macrophages markers.[34] Luu et al. used Ti with 400–500 nm wide grooves, on which macrophages were elongated along the direction of the patterns and showed increased levels of antiinflammatory IL-10 secretion and decreased secretion of TNF-α.[63] Arnold et al. fabricated nanoscale cell adhesive patterns with different spacing (50–250 nm) using PEG and RGD modified Au nanoparticles and observed increased projected cell area of MC3T3 osteobalsts in smaller gapping groups.[77] Despite many studies regarding the effect of various topographical features on cell behavior, their role in osteoimmunomodulation is not yet fully understood and requires further investigation.

Pores of implants are also critical in clinical use due to infiltration of biological molecules, including proteins or oxygen. In addition, these also affect cellular behavior, acting as topographical cues. In that sense, the frequency and sizes of the pores may act as important cues for osteogenesis and immunomodulation. Cells can differently interact with their microenvironment depending on the scales of the material, i.e., nano, micro and macro scale.[78] Even though differences in cell type can lead to different results, nanopores with diameters larger than 100 nm showed decreased cell adhesion.[79] Chen et al. introduced nanoporous structures into anodic alumina and demonstrated that surfaces with pores in diameters of 100 and 200 nm showed macrophages with round shape and increased expression of M2 phenotype markers. These results are contradictory to those from previous research on the effect of cell shapes on macrophage polarization.[34,80] On the other hand, Sussman et al. investigated the effect of microsized pores on macrophage polarization using poly(2-hydroxyethyl methacrylate) (pHEMA) and poly(methyl methacrylate) (PMMA). The materials with the pore sizes of 34 μm showed increased levels of M1 phenotype marker expression, including iNOS and IL-1R1 upon implantation into mice.[64] Garg et al. used porous polydioxanone (PDO) scaffolds with different pore sizes and porosities. The culture of mouse primary macrophages on the scaffold showed that the larger pore size and higher porosity group increased the expression of M2 macrophage marker arginase.[45] Despite the influence of pores on control over cell behaviors, the effects of the pore size are still controversial in regulation of osteogenesis and immune response, which should be further investigated in the future.[79]

3.2.3. Delivery of Biological Molecules for Osteogenesis

Biomaterials tailored to deliver various types of chemokines have been actively investigated to modulate cross-talk between immunomodulatory cells and osteoblasts. However, caution should be exercised when selecting bioactive molecules since cross-talk between immune cells and osteoblasts is complex, and several signaling pathways have been fully revealed with a number of pathways yet to be elucidated. Nonetheless, studies on osteoimmunomodulation by the delivery of biological molecules have been widely pursued. For example, Guihard et al. reported that an IL-6 family cytokine, Oncostatin M, induced osteoblast differentiation via signal transducer and activator of transcription (STAT) signaling.[65a] Loi et al. cocultured pre-osteoblasts and macrophages to investigate cross-talk between these two types of cells. Treatment of IL-4 cytokine favored polarization of macrophages into the M2 phenotype, and increased secretion of Oncostatin M was observed, suggesting an increase in the differentiation of osteoblasts.[65b] In addition, delivery of IL-1RA showed a positive effect on osteoblast differentiation via M2 polarization of macrophages.[65c] Although anti-inflammatory macrophages enhance bone formation, a synergistic effect between M1 and M2 may be needed for improved bone formation. Spiller et al. prepared a system to deliver different cytokines sequentially to macrophages by modification of decellularized bone scaffold with a short release of IFN-γ and sustained release of IL-4, which induced polarization of M1 and M2 phenotypes, respectively. The combined M1 and M2 phenotype induction leads to increased osteogenic properties by increasing the growth factor secretion of the macrophages (Figure 3d).[55] In one study, Kim et al. mimicked the osteoimmunomodulatory effect of cytokines on osteogenesis via rhIL-1ra-ELP fusion protein prepared by transforming *Escherichia coli*. The prepared protein was then immobilized on the surface of a self-assembled monolayer made of Cr and Au via carboxyl groups on the surface, which attenuated proinflammatory cytokines to enhance osteogenesis.[81]

Delivery of proteins also showed increased osteogenesis both for bone formation and for immunomodulation. Wei et al. introduced BMP-2 to mouse subcutaneous tissue for immunoregulation using a gelatin sponge. The results showed that BMP-2 delivery proved to be useful as both a direct inducible factor for osteogenesis and a passive control for osteogenesis via immune suppression.[66] Nucleic acids can also be used for osteoimmunomodulation. Li et al. used long noncoding ribonucleic acid (RNA) MALAT1 to MSC

culture. MSC showed an increased level of indoleamine 2,3-dioxygenase (RNA), which induced M2 macrophage polarization, and these particular groups finally enhanced osteogenic differentiation of MSCs.[67] Although there have been few (or no) attempts to directly load genes into scaffolds for osteoimmunomodulation, gene-activated matrices that contain a polycation–plasmid complex inside porous structure can be used.[82] There have been various approaches to modulate inflammation to control osteogenesis including delivery of cytokines, proteins or nucleic acid. Although many approaches have focused on reducing inflammation to enhance osteogenesis, the introduction of proinflammatory factor at the proper time also showed improved bone regeneration, and several proinflammatory cytokines (such as IL-6) can sometimes induce osteogenesis.[55,65a] Furthermore, some osteoinductive molecules such as BMP-2 are involved in complex signaling processes.[66] Thus, it is important to understand osteoimmunology and to carefully choose biological molecules with biomaterial carriers to achieve desirable results for each application.

4. Biomaterials for Modulation of Osteoclastogenesis

4.1. Immune Response and Osteoclastogenesis

Bone regeneration is a complex process, in which bone formation and resorption is balanced. Bone resorption is a two-step process that starts with the proliferation and differentiation of immature osteoclast precursors into osteoclast phenotypes and continues with the degradation of the organic and inorganic phases of bone.[83] Specifically, osteoclasts are polarized, form a "ruffled membrane," and secrete resorptive organelle transporting acidifying vesicles.[84] At the same time, they also generate an isolated extracellular microenvironment between themselves and the bone surface called "sealing zone." This structure is organized as a ring surrounding the ruffled membrane and linking matrixrecognizing integrins to the cytoskeleton.[85] The appearance of the membrane is evidence of bone resorption. In this zone, bone demineralization occurs by acidification of bone matrix mediated by a vacuolar H^+-adenosine triphosphatase on the surface of the osteoclasts, which secrete HCl into the zone. This acidic milieu (pH of ≈4.5) first mobilizes bone mineral. Then, the demineralized organic component of bone is degraded by a lysosomal protease and cathepsin K. The products of bone degradation are endocytosed by the osteoclast and transported to and released at the cell's antiresorptive surface.[83]

Direct contacts between osteoclasts and osteoblasts using receptor–ligand interactions have important roles in bone remodeling. For example, the ephirn B (EphB) receptor–ligand interactions activate bidirectional signaling. The bidirectional signaling could regulate the remodeling process by switching from bone resorption to bone formation.[86] Furthermore, the osteoblasts express a 317 amino acid peptide (a member of the TNF superfamily) called a receptor activator of nuclear factor kappa-B ligand (RANKL) on the bone surface. Then, the RANKL interacts with a receptor on osteoclast precursors called receptor activator of nuclear factor kappa-B (RANK). The RANKL–RANK interaction results in activation, differentiation, and fusion of hematopoietic cells of the osteoclast lineage so that they begin the process of resorption and also prolongs osteoclast survival by suppressing apoptosis. As a reverse step, the effects of RANKL are blocked by osteoprotegerin (OPG), a secretory dimeric glycoprotein belonging to the TNF receptor family. The OPG acts as a decoy receptor (acting as an antagonist) for RANKL and it is mainly produced by the osteoblasts or the other cells in the bone marrow. Thus, the OPG regulates bone resorption by inhibiting the final differentiation and activation of osteoclasts, and inducing their apoptosis.[87]

A number of reports demonstrated that osteoclastogenesis or the process of aforementioned ligand-receptor bindings are regulated by diverse biological factors secreted from osteoblasts and osteoclasts or other cells in bone marrow. First of all, TNF-α was reported to induce the differentiation of osteoclasts from bone marrow macrophages and also induce in vivo bone resorption.[88] However, TNF-α alone does not induce osteoclast differentiation because the effect of TNF-α was found in osteoblast and osteoclast cocultured platform. The TNF-α enhanced IL-1 expression, which induces RANKL expression on stromal cells.[89] The IL-1 also stimulates osteoblasts to produce M-CSF which inhibit osteoclast apoptosis in a dose-dependent manner and decrease osteoblastogenesis via modulation of mitogenactivated protein kinases (MAPK). This causes downregulation of bone formation and stimulates Dickkopf-related protein 1 and sclerostin, which may suppress osteoblast differentiation via inhibition of Wnt signaling.[90] Next, the IL-6 can significantly enhance bone resorption by increasing the expression of RANKL.[91] IL-11 and LIF produced from bone cells can stimulate osteoclastogenesis and bone resorption in response to resorption stimuli such as parathyroid hormone (PTH).[92] Both IL-15 produced by T cells and monocytes from the peripheral blood enhances osteoclast differentiation and bone destruction. A previous study reported that IL-15 promoted osteoclast formation by significantly upregulating the expression of RANKL and phospholipase D-1 (PLD1) via activation of the MAPKs and NF-kB pathways.[93] In addition to these cytokines, various small cytokines specifically, IL-8,[94] CXCL12,[95] CCL2,[96] and CCL9[97] also enhanced osteoclastogenesis and bone resorption.

In contrast, some cytokines have anti-osteoclastogenic effects. IL-10 produced by activated T and B lymphocytes inhibits bone resorption via downregulation of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) expression, which is needed for osteoclast differentiation. IL-10 also downregulates osteoblastogenesis by decreasing the expression of bone proteins such as alkaline phosphatase (ALP) and collagen type 1, and inhibits mineralization of the bone matrix.[101] Likewise, suppressing IL-17 induced both RANKL expression in osteoblast and RANKL-responsive osteoclast differentiation.[102] The exact mechanism of how to IL-18 decreases osteoclastogenesis is somewhat controversial in that one study demonstrated that increased IL-18 produced by osteoblast inhibits TNF-α-mediated osteoclastogenesis.[103] However, another study using IL-18 overexpressed in a transgenic mouse proved that net effects promoted osteoclast formation and resorption.[104] Lastly, IL-12 inhibits RANKL-mediated osteoclastogenesis when cocultured with osteoblastic cells, and also inhibits TNF-α stimulated osteoclast differentiation.[103,105] In conclusion, various proinflammatory cytokines are related to osteoclastogenesis and regulate the process of bone resorption (**Figure 4**a). However, the role of the majority of other cytokines in osteoclastogenic behaviors is still unclear or controversial.

4.2. Modulation of Osteoclastogenesis Using Biomaterials

Although using biomaterials for enhancing bone regeneration has been studied for long time, activation or deactivation of osteoclastogenesis as a key modulator of bone regeneration and remodeling has only been highlighted recently.[120] Furthermore. lots of biomaterials have been studied to regulate osteoclastogenesis for bone remodeling as shown in **Table 2**. In these approaches, it is important to choose whether up or downregulation of the osteoclastogenesis is required depending on the situation. For examples, defects such as fracture need rapid new bone formation and refined lamella structured bone, and thus, implants would activate both osteoblastogenesis and osteoclastogenesis.[121] However, bone diseases from genetic disorders, hormone imbalance and aging such as osteoporosis would require strategy to deactivate osteoclastogenesis while enhancing bone formation because patients with the disease already have highly activated osteoclasts or weak bones.[122]

4.2.1. Surface Chemistry of Biomaterials

Modification of surface chemistry can be employed to regulate the function of osteoclasts. First of all, an increase in hydrophilicity of the material may decrease osteoclast activity. Bang et al. seeded bone marrow-derived macrophages on two different types of titanium surfaces, in which the one was sandblasted/ acid etched and the other was hydrophilic sandblasted/acid etched titanium. The macrophages were cultured in the presence of RNAKL and M-CSF for osteoclastic differentiation. All osteoclastic markers such as tartrate-resistant acid phosphatase (TRAP), osteoclast-associated immunoglobulin-like receptor (OSCAR), MFATc1, and c-Fos were

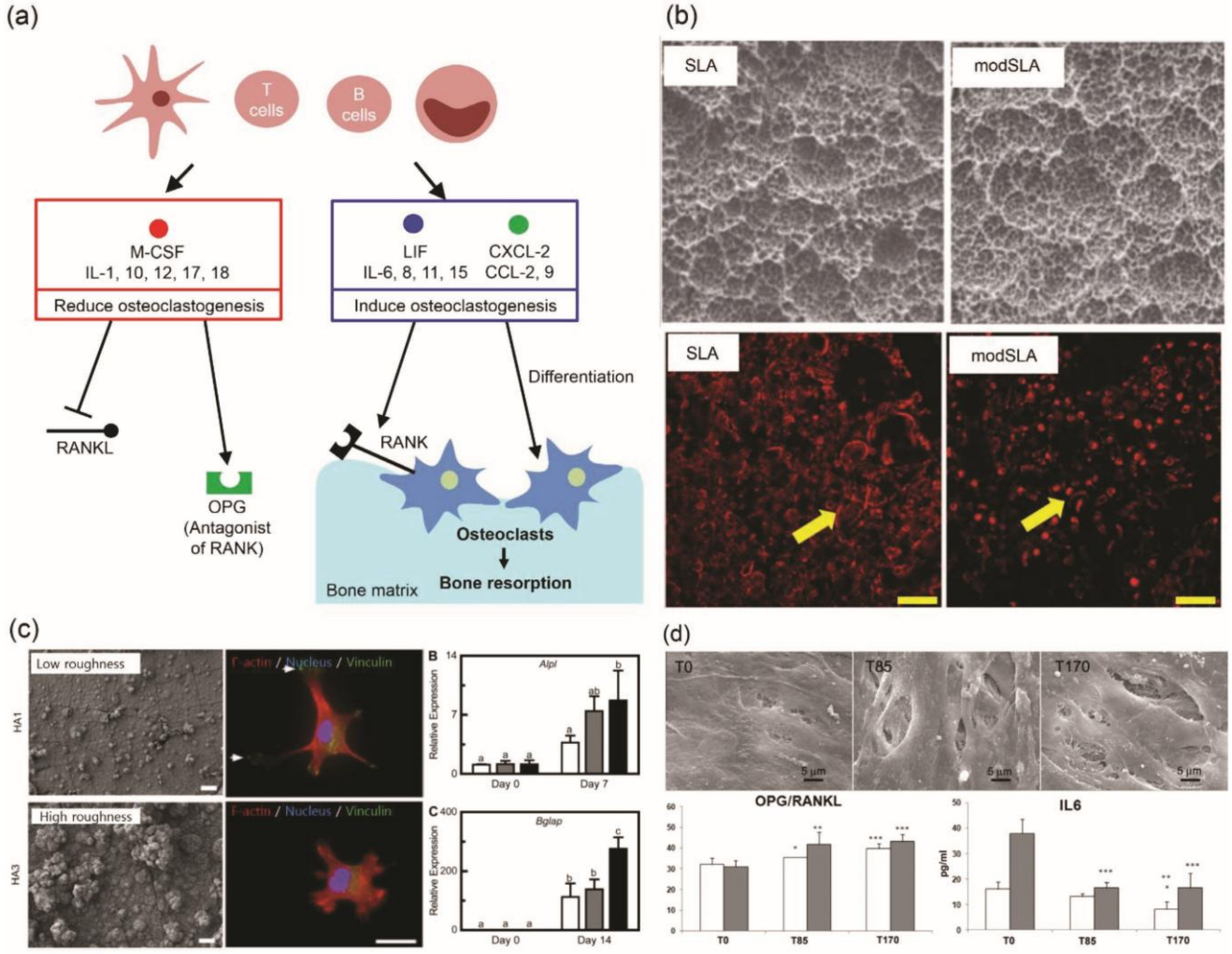

Figure 4. a) Cross-talk among immune cells, osteoblasts, and osteoclasts during bone healing processes. b) SEM images of sandblasted/acid etched (SLA) and hydrophilic-modified SLA (modSLA) surfaces of titanium and osteoclasts attachment to each surfaces. Reproduced with permission.[98] Copyright 2014, Elsevier. c) SEM images of HA coating with different levels of roughness and morphology/focal adhesion structure of osteoclasts attached on the corresponding surfaces, and their gene expression of alkaline phosphatase (Alpl) and osteocalcin (Bglap). Reproduced with permission.[99] Copyright 2013, Elsevier. d) The different concentration of quercetin functionalized HA materials were prepared in trans-well. The osteoblasts were seeded on the HA and the osteoclasts were seeded on the trans-well. The images were the SEM images of osteoblasts on the different materials and secreted amount of OPG and IL-6 from osteoblasts was illustrated. Reproduced with permission.[100] Copyright 2016, Elsevier.

downregulated on the hydrophilic surface (Figure 4b).[98] Also, the modification of surface with Mg^{2+} could suppress osteoclastogenesis. Bose et al. demonstrated that doping of magnesium ions on β-TCP, main component of bone crystal and resorbed by osteoclasts, reduced bone resorption. Experiments with osteoclast precursor RAW264.7 cells cultured on normal TCP substrate or Mg^{2+} immobilized TCP surface showed that the cells cultured on the Mg^{2+}-immobilized surface were not differentiated into osteoclasts with mononucleated features and did not show actin ring formation.[106]

Also, the chemical composition of bioceramics can affect osteoclastogenesis. The calcium phosphate (CaP) ceramics are the most commonly used materials for bone regeneration. These include several materials with different chemical compositions and crystal phases. Among them, the two most studied CaP ceramics are β-TCP and hydroxyapatite (HA). The β-TCP degrades too fast due to high solubility. On the other hand, HA is essentially insoluble and its degradation is only limited to the surface by osteoclasts since cells are not able to breach the microporous ceramic arrangement. biphasic calcium phosphate (BCP), a mixture of HA and β-TCP with varying ratios, may provide an optimal formulation in terms of bone resorption and final degradation.[107] For example, Yamada et al. investigated osteoclastic resorption on HA substrates, β-TCP substrates, and two types of BCP substrates in different HA/β-TCP ratios of 25/75 and 75/25 for two days using neonatal rabbit bone cells. The results demonstrated that BCP with an HA/β-TCP ratio of 25/75 exhibited the most extensive resorption by osteoclasts. Smaller discontinuous island-like lacunae were observed on the β-TCP substrates. No resorption lacunae was found on HA or BCP with an HA/β-TCP ratio of 75/25.[108] Similarly, Wepener et al. created biphasic HA (40%)/β-TCP (60%) nanoscaffolds by electrospinning and then cultured human osteoblasts (hFOB 1.19) and monocytes (THP-1). In this study, both cell lines showed no cytotoxicity, decreased apoptosis and well differentiated osteoclast-like cells.[109] In another study, Botelho et al. demonstrated that silicon incorporation also affected osteoclastogenesis. They cultured osteoclasts isolated from peripheral mononuclear blood cells (PBMCs) on 1.5 wt% Si dissolved HA ceramic substrates that showed higher osteoclastic activity compared to the cells on unmodified HA.[110] Collectively, the osteoclastogenesis can be

Table 2. Biomaterial strategies for modulation of cross-talk between immune response and osteoclastogenesis.

Engineering parameters	Property		Regulatory effect on immune response		Effects on osteoclasts	Ref.
			Upregulation	Downregulation		
Surface chemistry	Hydrophilic surface				(−)	[98]
	Ions (Mg^{2+})				(−)	[106]
	HA contents				(+)	[107–110]
Topography	Roughness	1–10 μm	Sealing zone formation, osteoclast differentiation		(+)	[111,99,112,113]
		>10 μm	TRAP activity	Apoptosis of osteoclasts	(+)	[114]
		<1 μm		Osteoclast differentiation	(−)	[99]
Deliver of chemicals	Alendronate		Apoptosis of osteoclasts		(−)	[115,116,100,117]
	Quercetin			IL-6	(−)	[100,117]
	PVPA-AA		OPG		(−)	[118]
	RSV			RANKL	(−)	[119]
Deliver of biomolecules	Proteins	OPG		RANKL	(−)	[87,103]
		M-CSF		Wnt signaling, apoptosis of osteoclasts	(+)	[90]
		TNF-α	RANKL		(+)	[88,89]
	Cytokines	IL-6	RANKL		(+)	[91]
		LIF	PTH		(+)	[92]

regulated by chemical modification of substrates, and osteoclastic differentiation may be downregulated on hydrophilic surfaces. In contrast, various methods for changing the chemical composition of bioceramics have been investigated to enhance osteoclastic activity. The results of the previous studies seem to be a little controversial, however, the ratios of HA, β-TCP and additives may be important when regulating osteoclast activities.

4.2.2. Topography of Biomaterials

Surface topography modification can also be used to regulate various cell functions. Specially, modifying roughness of dental implants has been used to regulate osteoblastogenesis and osteoclastogenesis and to increase rapid bone integration with the implanted substrate. In one study, researchers changed the macrostructure of a biphasic calcium phosphate (BCP, 80% of HA and 20% of TCP) substrate and investigated the response of osteoclast precursor cells (RAW264.7). BCP1150 with a small surface microstructure (≈1 μm) formed ectopic bone adjacent to multinucleated osteoclast cells in the muscles of dogs. The implants were in the form of planar discs so macro-scale features such as concavities, macropores and interparticle spaces were unnecessary for this response. In contrast, BCP1300 with identical compositional chemistry but larger surface architecture (≈2–4 μm) formed neither osteoclast-like cells nor ectopic bone.[111] Costa et al. also studied the reaction of osteoblasts and osteoclasts when the cells were seeded on PCL surfaces with different topographical features. They coated PCL using different concentrations of HA for synthesizing three different substrates with different roughnesses. In conclusion, authentic osteoclasts isolated from the long bones of neonatal New Zealand white rabbits cultured on a microrough surface (HA3: Ra = 363 nm, 2.0 μm roughness) showed disrupted F-actin sealing zones that made osteoclasts fail to resorb on HA surfaces while the osteoclasts resorbed on the surfaces with microscale roughnesses (HA1: Ra = 194 nm, 1.0 μm roughness, HA2: Ra = 316 nm, 1.3 μm roughness) (Figure 4c).[99]

Furthermore, various methods for the modification of titanium surface roughness by polishing and sandblasting have been actively studied for rapid regeneration of dental disorders.[113,123] For example, Shemesh et al. seeded osteoclasts (RAW264.7) on a longitudinal bone slice exhibiting a rough surface or the same bone slice with a smooth surface to observe the different activities of osteoclasts. The osteoclasts on the rough surface showed a larger sealing zone ring diameter (≈15 μm) compared with the ring on a smooth surface (≈8 μm) and the life span of the sealing zone was longer on a rough surface (≈80 min).[114] Sommer et al. used titanium, TiAl6Mo7, CoCr28Mo6, and FeCrNi substrates with polished and sandblasted surfaces to evaluate osteoclastogenesis. The results demonstrated that the TRAP activity and the number of osteoclasts were higher on the sandblasted surfaces (rough) than on the polished surfaces (smooth), while different metal alloys did not affect osteoclastogenesis significantly.[112] Similarly, RAW264.7 cells cultured on titanium substrates with smooth (TS), acid-etched (TA), and sandblasted/acid etched (SLA) surfaces revealed that osteoclasts on rough surfaces (TA and SLA) showed similar osteoclastogenesis with those on native bone, whereas limited osteoclastogenesis was observed on smooth surfaces.[113] Taken together, the results indicated that nano/microscale surface architecture may be a crucial parameter in regulating osteoclastogenesis; however, the exact mechanism still needs to be fully elucidated.

4.2.3. Delivery of Biological Molecules for Modulation of Osteoclastogenesis

As previously shown, biomaterials have been utilized as a carrier for delivery of biological molecules, various chemicals and drugs, and osteoclastogenesis is influenced by these factors.[124] Recently, various approaches have been studied for synthesizing functionalized substrates that regulate the activity of osteoclasts, especially for treatment of osteoporosis. The most common approach was to use alendronate, one of the most popular anti-osteoporosis drugs. For example, Lee et al. immobilized alendronate on the surface of gold nanoparticles (GNPs), showing enhanced bone regeneration.[115] Specifically, 0.204×10^{-3} M of the alendronate was conjugated with GNPs (GNPs– ALD) and then treated in in vitro cultured bone marrow derived macrophages (BMM) from an ICR mouse and in vivo in a femur bone of a mouse. The study revealed that the activity of osteoclasts significantly decreased compared with normally cultured cells. Furthermore, double the trabecular bone volume/ tissue volume (%) of the femur bone was regenerated, and the quantity was similar to that of the host bone.[116] Also, Boanini et al. coated mesoporous glass nanospheres with 56×10^{-3} M of alendronate to reduce osteoclast activity.[100] Another approach involved the use of the antioxidant quercetin. Boanini group's investigated the effect of quercetin by conjugating the molecules to the HA substrate and demonstrated that the conjugated quercetin successfully reduced the release of inflammatory cytokines (such as IL-6) and the activity of human osteoclast precursor cells T-110 (Figure 4d).[100] Furthermore, Forte et al. functionalized HA substrate with quercetin and alendronate together. Then, 56×10^{-3} M of alendronate and $10–100 \times 10^{-3}$ M of quercetin were stably immobilized on an HA substrate and showed a sustained release profile (7% released after 160 h). A culture of human osteoblast-like cells (MG63) and human osteoclast precursor (T-110) on the functionalized HA showed decreased osteoclast viability and differentiation capacity.[117]

Electrospun fibers have been studied for long time as promising scaffold materials for bone regeneration since the morphology of the fibers successfully mimics the collagen fibrils of bone tissue and the fibers effectively receive biological molecules by dissolving or surface immobilization.[125] Recently, Downes et al. used a novel polymer, poly(vinyl phosphonic acidco-acrylic acid (PVPA-AA), to synthesize electrospun fibers. Cultures of osteoblasts (human osteoblast cells, HOBs) and osteoclasts (human osteoclast precursor cells) on these substrates over 14 days successfully induced differentiation, maturation and mineralization of HOBs while reducing the number of viable osteoclasts by inducing apoptosis of the cells because the PVPA-AA increased the expression of OPG from osteoblasts.[118] Similarly, Riccitiello et al. used RSV (a drug used to influence bone osteogenesis by decreasing RANKL mediated osteoclastogenesis) to prepare electrospun nanofibers. The fiber showed sustained release of resveratrol (RSV) and dental pulp stem cells (DPSCs) and expressed less RANKL while osteoclast precursors were unable differentiate into mature

osteoclasts.[119] Collectively, the approaches using functionalized biomaterials for regulating osteoclastogenesis have still been limited to several molecules, however, the role of various biological factors regulating the osteoclast activity has become clearer. Thus, more studies investigating the regulation of osteoclastogenesis by using functionalized biomaterials with these molecules may be promising for next generation bone engineering strategies.

5. Biomaterials for Modulation of Infection and Inflammation

5.1. Inflammation-Mediated Infection of Biomaterials

The ideal biomaterial for orthopedic implants should have high biocompatibility and reproducible functions such as osseointegration, i.e., stimulating regeneration of new bone.[126] A great number of studies have been dedicated to improve the biomaterial properties for orthopedic implants, and these have particularly focused on osseointegration and regenerative functions.[126b] However, the biofilm formation induced by bacterial adhesion is a critical problem in biomedical implants and devices. Undesirable biofilm formation on the surface of implanted devices generally results in implant failure that can be life threatening.[127] Furthermore, secreted factors from biofilms such as LPS, lipoprotein, lipoprotein acid (LTA) or peptidoglycans induce inflammatory responses for mammalian immune cells that lead to implant failure by a systemic defense reaction. For example, Arima et al. demonstrated that secreted factors from *Staphylococcus pseudintermedius* biofilms induce an immune response on RAW264.7 cells while upregulating the expression of inflammatory cytokines such as IL-1β and IL-6.[128] Therefore, understanding the relationship between bacterial infection on implanted materials and immune response is important.

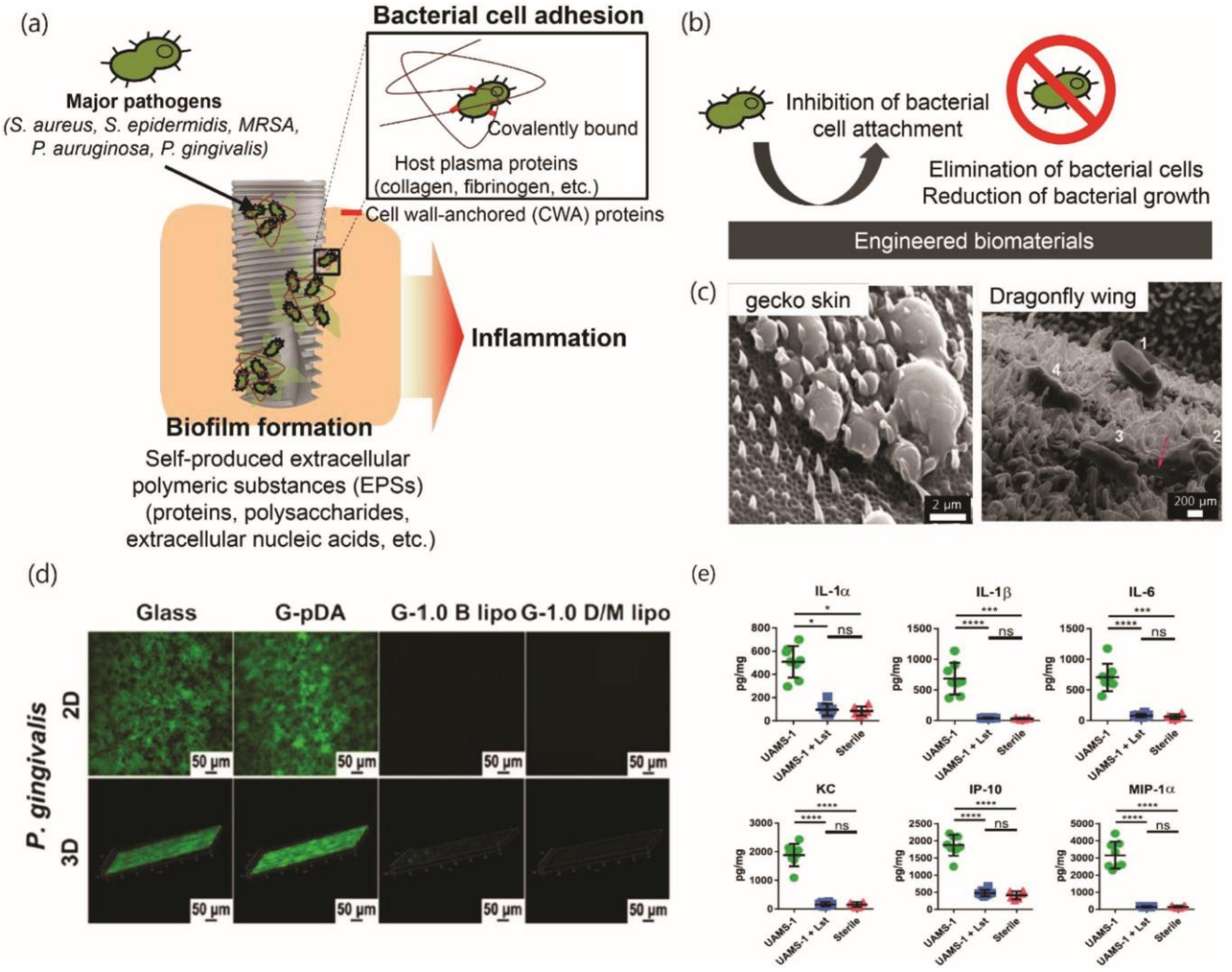

Figure 5. a) Schematic illustration of biofilm formation on the surface of implant biomaterials caused by inflammation and infection. b) Schematic illustration of the prevention of biofilm formation by engineered biomaterial surface. c) SEM images of the morphology of bacteria attached on the biomimetic surface presenting nano- and microstructure: *P. gingivalis* on gecko skin. Reproduced with permission.[129] Copyright 2017, American Chemical Society. *E. coli* on dragonfly wing. Reproduced with permission.[130] Copyright 2016, Royal Society of Chemistry. d) Prevention of the *P. gingivalis* biofilm formation by the surface functionalized with liposomal minocycline. Reproduced with permission.[131] Copyright 2017, American Chemical Society. e) Secretion of proinflammatory cytokines including IL-α, IL-1β, IL-6, KC, IP-10, and MIP-α was downregulated on the implant surface functionalized with PEG hydrogel loaded with lysostaphin. Reproduced with permission.[132] Copyright 2018, United States National Academy of Sciences.

Furthermore, developing strategies for anti-infection and antiinflammation activity is important to overcome implant failure.

In trauma, skin commensal bacterium such as *staphylococcus aureus* and *Staphylococcus epidermidis* are the most common pathogens. In the case of peri-prosthetic joint infections (PJI), the most common pathogens are again *S. aureus* and *S. epidermidis*, but methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative *Pseudomonas aeruginosa* may be included (**Figure 5**a). When the bacterial cells attach on the implant surface, the biofilm development processes begin and proceeds in four stages: a) initial bacterial attachment, b) generation of a multilayer of bacterial cells through cell accumulation and aggregation, c) biofilm maturation and matrix elaboration, and d) bacterial migration for new biofilm cycles in different locations.[126b] Bacterial cells have diverse mechanisms to adhere to foreign material surfaces and host tissues. Once bacterial attachment occurs, the formation of a small colony of initial cells occurs, followed by biofilm formation via self-produced extracellular polymeric substances (EPSs) and cell proliferation. EPSs are composed of proteins and polysaccharides, and extracellular nucleic acids protect the bacterial cells from immune cell invasion and antimicrobial serum factors while providing mechanical stability to the bacterial environment (Figure 5a).[133]

Bacterial attachment on material surface or host tissue is important in the initial stage of biofilm formation. As previously mentioned, bacterial cells have diverse adhesion mechanisms. For example, *S. aureus*, which is the most common pathogen in PJI expresses cell wall-anchored (CWA) proteins (Figure 5a). The CWA proteins, which make covalent bonds with a pathogen's peptidoglycan layer, are classified into four groups: a) the microbial surface component that recognizes the adhesive matrix molecule (MSCRAMM) family, b) the near-iron transporter (NEAT) motif

family, c) the three-helical bundle family, and d) the G5-E repeat family.[134] Those CWA proteins can initiate attachment on host plasma proteins such as collagen, fibrinogen, keratin, and elastin.[135] Then, *S. aureus* starts to secrete immunoevasive proteins. Various proteins such as hemolysins, leukocidins, enzymes, phenol-soluble modulins, and chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS) play roles as immunoevasive proteins to lyse neutrophils, monocytes and macrophages, degrade immune proteins, and inhibit of neutrophil migration.[135]

Macrophages and neutrophils exhibit the first line of defense against bacterial infection using oxidative bursts, production of bactericidal peptides and proinflammatory cytokines.[136] M1 macrophages induce proinflammatory responses such as IL-1β, TNF-α, IFN-γ secretion and production of iNOS against *S. aureus* to promote bacterial clearance. Staphylococcal biofilms actively avoid recognition by TLR2 and TLR9 and skew macrophage attacks towards an alternative activation of antiinflammatory M2 macrophage.[137] In conclusion, the biofilm critically affects immune cells and leads to an immune response that can reduce the bone tissue regeneration that determines the success rate of orthopedic implants.

Immune responses in the implanted material involve the secretion of inflammatory cytokines, which play an important role in bone regeneration. Johnson et al. engineered injectable PEG hydrogels that deliver lysostaphin, which is a bacteriolytic enzyme. In a *S. aureus* infection in bone fracture, lysostaphinreleasing hydrogel successfully eradicated *S. aureus* biofilm and reduced the inflammatory environment to support fracture healing. The ward method of hierarchal clusters revealed that inflammatory cytokines such as granulocyte colony stimulating factor (G-CSF), IL-α, IL-1β, IL-6, keratinocyte chemoattractant (KC), IP-10, macrophage inflammatory proteins (MIP)-1α, MIP1β, and MIP-2 were significantly enhanced in infectious conditions, whereas lysostaphin-PEG hydrogel treated groups were downregulated to the level of sterile groups (Figure 5e). Finally, the lysostaphin-PEG hydrogel group fully healed in 5 weeks with bone formation and restored mechanical properties in a murine femur fracture model.[132] Moreover, periodontal pathogens closely related to dental implants are also directly related to inflammation factors. Kim et al. investigated anti-inflammatory reactions of the magnoliae cortex and maize with inflammation reactions induced by *Porphyromonas gingivalis*. In an infection with *P. gingivalis*, RAW264.7 cells exhibited high expression of inflammatory factors such as IL-1β, IL-6, and nitrite products. However, the magnoliae cortex and maize-treated group showed decreased levels of those inflammatory factors, indicating that it had a positive effect on infection-related inflammation.[138]

5.2. Modification of Biomaterials for Modulation of Infection and Inflammation

Researchers have considered the importance of bacterial infection and inflammation reactions on orthopedic implants. Thus, anti-infection and anti-inflammation strategies have been actively investigated, and the concept of immunomodulation for bone tissue engineering began to emerge and be applied to implantable biomaterials. Treatment of bacterial infection requires various strategies. Surgery is usually needed to remove biofilm-coated implants accompanied by systemic antibiotic drug administration. However, high doses of antibiotics have side effects and might negatively affect osteogenesis.[139] Recently, researchers have widely utilized the surface modification of orthopedic implants, which can provide clinical advantages. For example, prevention of bacterial infection in the early stages reduced the dose of antibiotics resulting in low cytotoxicity and improved clearance of infection.[126b] Various modification strategies on orthopedic implant such as modification of surface chemistry, topography, immobilization, or coating methods using biomolecules and chemicals have been investigated for bone tissue engineering applications (Figure 5b). In addition, modulation of inflammation and immune response, which are deeply related to the bone healing process, has been widely investigated. As recent studies have revealed how immune systems affect bone repair or regeneration, researchers have widely investigated modification strategies more focused on the role of anti-inflammation (including modification of surface properties and the delivery of vaccines or antiinflammatory molecule). In this review, we summarize the recent trends in antimicrobial, anti-inflammation strategies that can potentially be used for clinical applications of orthopedic implants.

5.2.1. Surface Properties of Biomaterials

In the last decade, researchers have been interested in eliminating bacteria by altering the physical topography of biomaterial surface, rather than chemical treatment of the surface of implant materials. It has been postulated that the bacterial cell walls disfigure and stretch when they interact with surfaces presenting certain levels of texture; thus, cell rupture and death may occur.[140] Surface roughness and topography of the implant materials significantly affected hydrophobicity, van der Waals forces, electrostatic interactions, and steric hindrance, which are factors that determine bacterial attachment.[141] Nano- and microstructures significantly increase the surface area, which may create more effective bactericidal activity than flat surfaces. Furthermore, bactericidal activity of the biomaterial surface may be related with structural spacing, radius and height.[142] For example, antimicrobial strategies that employ nano- and micro structures of biomimetic surfaces such as lotus leaves, shark skin, cicada and dragonfly wings revealed that biophysical cues of biomaterials are highly related to bacterial clearance, which can be collectively applied to implant biomaterials (Figure 5c).[129,143]

The use of surface biophysical modification for implant antiinflammation and immunomodulation activity has also been widely investigated. For example, Pan et al. investigated the immunomodulation properties of a hierarchical macropore/nanosurface. In the hierarchical surface, macrophages were switched to M2 phenotype, decreasing the expression of inflammatory genes as well as upregulating anti-inflammatory genes. The authors suggested that this may be related to the cytoskeleton tension induced by certain cell shapes. In addition, osteogenic differentiation of MSCs and angiogenesis of HUVECs was upregulated in a RAW264.7 cells/hierarchical surface conditioned medium, which were expected by enhanced expression of BMP-2 and VEGF of RAW264.7 cells.[144]

Osseointegration is one of the most important parameters that directly determines the success or failure of implantation. The process of osseointegration requires both interaction between angiogenesis and osteogenesis, and an immune microenvironment. Bai et al. investigated the influence of microporous Ti surfaces coated with nanoparticles versus nanorods of HA on osseointegration and multiple cell behavior. The results showed that the Ti surface coated with nanoparticles of HA positively modulated inflammation resulting in an osteoimmune microenvironment more favorable for angio/osteogenesis via activation of key signaling proteins (TGF-β, OPG, RANKL, and VEGF). However, the Ti surface coated with nanorod-shaped HA particles exhibited a negative effect on the same processes. In vivo results also confirmed that nanoparticles of HA exhibited higher levels of new bone formation and osseointegration compared to the nanorod group.[151] The recent trends of implant modification with biophysical cues for antimicrobial and anti-inflammation strategies are summarized in **Table 3**.

5.2.2. Surface Modification of Biomaterials with Bioactive Molecules

A number of studies have been carried out focusing on bone implant modification with bioactive molecules with antimicrobial and anti-inflammation ability such as antibiotics, metal ions, peptides, vaccines, hydrogels, and heparin via coating or immobilization chemistry.[62b,131,152,154,157-159] First, the most promising antimicrobial molecules are antibiotics. To enhance the efficacy of antibiotics, local delivery using immobilization methods has been widely investigated. Li et al. applied antibiotic-loaded polymeric coating on a Ti implant to prevent bone infections. PEG-based hydrogel films with vancomycin were covalently bound to the Ti implant. Sustained release of vancomycin from the hydrogel for nearly 3 weeks in vitro and 4 weeks in vivo confirmed the antimicrobial activity in a rabbit infection model with *S. aureus* while significantly reducing the inflammatory reaction.[131] Furthermore, Xu et al. suggested a dual functional implant surface with the capacity to reduce nonbacterial inflammation from implanted materials and improve antimicrobial properties. They coated a polystyrene culture plate with dexamethasone with minocycline-loaded liposome via polydopamine chemistry. Liposomal dexamethasone reduced the expression of proinflammatory factors such as IL-6 and TNF-α in human mesenchymal stem cells and LPS-stimulated human gingival fibroblasts. Furthermore, the liposomal minocycline prevented bacterial adhesion and proliferation of *P. gingivalis* (Figure 5d) and *Streptococcus mutans*.[131] Silver nanoparticles have been widely used for antimicrobial activity in various fields, in which silver ions break through the bacterial cell wall and disrupt the respiration in mitochondria, attaching to the DNA to stop cell replication.[62b,152] In addition, various types of antimicrobial peptides (AMPs) derived from many proteins have been utilized, and novel applications in both physical immobilization of AMPs on the surface and chemical conjugation of AMPs have been widely investigated.[157–159]

Previous studies revealed that various biological and natural compounds can be used as anti-inflammatory molecules, which have advantages for reducing inflammatory response. For example, Kim et al. investigated the anti-inflammatory properties and osteoblast function of heparin and BMP-2 immobilization on Ti. As heparin has well characterized anticoagulant and anti-inflammatory properties, heparin and BMP-2 immobilized on the Ti exhibited significantly higher ALP activity and calcium deposition on MG-63 cells with decreased

Table 3. Biomaterial implants for modulation of immune response and infection.

Engineering parameters	Property	Antibacterial	Anti-inflammatory	Ref.
Surface chemistry	Zwitterions	(+)	(−)	[145]
	Polyelectrolytes	(−)	(+)	[146]
	Plasma treatment	(+)	(−)	[147]
Topography	Nanowires	(+)	(−)	[148]
	Nanopillars	(+)	(−)	[148]
	Macropore on nanosurface	(−)	(+)	[144]
	Brush structure	(+)	(−)	[149]
	Micro/nanostructure	(−)	(+)	[150]
	Nanoparticle/nanorod	(−)	(+)	[151]
Delivery of chemicals	Lysostaphin	(+)	(+)	[132]
	Silver nanoparticles	(+)	(−)	[62b,152]
	Citric acid	(+)	(−)	[127]
	Graphene oxide	(−)	(+)	[153]
	Forsythiaside	(+)	(+)	[154]
	Dexamethasone + minocyclin	(+)	(+)	[131]
Delivery of biomolecules	AMP	(+)	(−)	[155]
	Anti-inflammatory peptide	(−)	(+)	[156]
	Chimeric peptide	(+)	(−)	[157]
	RGD + lactoferrin	(+)	(−)	[158]
	Ti binding peptide + AMP	(+)	(−)	[159]
	Heparin + BMP-2	(−)	(+)	[160]
	Platelet proteins	(−)	(+)	[161]

inflammatory response. Furthermore, natural compounds such as forsythiaside, magnoliae cortex, and maize have also been used for similar applications.[138,154] The recent results of implant modification with molecules for antimicrobial and anti-inflammation strategies are also summarized in Table 3.

6. Conclusions and Future Perspectives

The success of biomaterials for bone regeneration is defined by how well biomaterials integrate with in vivo local bone microenvironments and regulate the key bone healing events. The response of the host immune system to biomaterial implantation is the main barrier that lies between the success of biomaterials and their applications in bone tissue engineering. Even though inflammation is the key event in the bone healing process, chronic inflammation due to foreign materials can also lead to failure of implants. In recent years, we have gained a better understanding of the biology of the pivotal cell types in the immune system, in particular, macrophages, neutrophils, and their interaction with biomaterials. Due to advances in biomaterial research, biomaterial design approaches has been shifted from traditional "immune friendly" to "immune reprogramming" biomaterials. Based on the careful review of existing data, we can broadly classify these efforts into surface modification of biomaterials with chemical approaches, physical modification, and functionalization with biological molecules. Chemical modification of materials with various functional groups, densities, or surface charges or modifications to improve the physical properties of the biomaterial (such as topography and stiffness) can be effectively used to modulate the functions of bone-forming cells and immune cells. Surface modification with biological molecules either in a surface decorated form or as delivering molecules also plays a major role in the current biomaterial-mediated immunomodulation approaches for bone tissue engineering.

Although the previously mentioned biomaterial-mediated approaches thus far have shown encouraging results in controlling inflammation in both in vitro and in vivo experiments, more extensive analysis should be pursued since the degradation products of the synthetic materials can exist in the body for a relatively long period of time. Moreover, macrophage polarization from M1 to M2 happens in a spatiotemporal manner, and biomaterial-mediated macrophage polarization has to be modulated given the fact that cytokines produced from M1 and M2 phenotypes have a very precise role in the bone regeneration process at specific time intervals. For example, an appropriate level of cytokines like TNF-α and IL-6 is critical during the initial stage of the healing process for balancing the immunity and recruitment of other immune cells and progenitor cells at the defect site. Hence, more extensive screening of type, density, and duration of signals may be needed to validate their effects on the macrophages. A high throughput screening method may be utilized in this regard and such studies should also extend to other critical cells in the immune system such as dendritic cells or T cells, and such information is very limited in the current literature. Moreover, although there are increasing evidence of effects of biomaterial properties on osteoblasts and macrophages, their influence on osteoclasts and osteoclasts precursors is relatively unclear. From the existing data, the osteoclastogenesis is highly influenced by cytokines from immune cells and osteoblasts; however, the majority of researches defined the effects of biomaterials in vitro culture and a very few papers have dealt with the reaction of osteoclasts in vivo environment when implanted with biomaterials. Furthermore, Surface modification strategy for anti-infection is focused on the bacterial attachment and removal while antiinflammation strategy is focused on the immune response of mammalian cells. However, these strategies ignored relationship between inflammation and bacterial effects and also most of the current bacterial infection model cannot provide critical evidence for practical infection process of bone implant. The gap between two different objectives makes it difficult to understand what exactly happens on the implanted materials and how the infection induce inflammation on implanted area. Therefore, appropriate models dealing with both antiinfection and anti-inflammation are essentially needed for further investigation. Moreover, it is also beneficial to design multifunctional materials that can both modulate the immune response and control other bone regeneration processes such as osteogenesis, osteoclastogenesis, or inflammation. This will help the bone tissue regeneration process reach the best functional outcome.

Acknowledgements

J.L. and H.B. contributed equally to this work. This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (Grant Nos. NRF2016R1A2B3009936 and NRF-2018M3C1B7021994).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

biomaterial interfaces, bone regeneration, macrophages, osteoimmunology

Received: September 4, 2018
Revised: September 19, 2018
Published online:

[1] S. N. Khan, F. P. Cammisa Jr., H. S. Sandhu, A. D. Diwan, F. P. Girardi, J. M. Lane, *J. Am. Acad. Orthop. Surg.* **2005**, *13*, 77. [2] M. M. Stevens, *Mater. Today* **2008**, *11*, 18.

[3] Z. Ai-Aql, A. S. Alagl, D. T. Graves, L. C. Gerstenfeld, T. A. Einhorn, *J. Dent. Res.* **2008**, *87*, 107.

[4] C. Schlundt, H. Schell, S. B. Goodman, G. Vunjak-Novakovic, G. N. Duda, K. Schmidt-Bleek, *J. Exp. Orthop.* **2015**, *2*, 1.

[5] a) M. Lind, B. Deleuran, H. Yssel, E. Fink-Eriksen,

K. Thestrup-Pedersen, *Cytokine* **1995**, *7*, 78; b) G. Mori, P. D'Amelio, R. Faccio, G. Brunetti, *Clin. Dev. Immunol.* **2013**, *2013*, 16.

[6] Y.-P. Li, P. Stashenko, *J. Immunol.* **1992**, *148*, 788. [7] N. Charatcharoenwitthaya, S. Khosla, E. J. Atkinson,

L. K. McCready, B. L. Riggs, *J. Bone Miner. Res.* **2007**, *22*, 724.

[8] S. Franz, S. Rammelt, D. Scharnweber, J. C. Simon, *Biomaterials* **2011**, *32*, 6692.
[9] J. Reifenrath, N. Angrisani, N. Erdmann, A. Lucas, H. Waizy, J. M. Seitz, A. Bondarenko, A. Meyer-Lindenberg, *Biomed. Mater.* **2013**, *8*, 045012.
[10] M. E. Bianchi, *J. Leukocyte Biol.* **2007**, *81*, 1.
[11] H. Kumar, T. Kawai, S. Akira, *Int. Rev. Immunol.* **2011**, *30*, 16.
[12] T. L. Adair-Kirk, R. M. Senior, *Int. J. Biochem. Cell Biol.* **2008**, *40*, 1101.
[13] a) A. Ozinsky, D. M. Underhill, J. D. Fontenot, A. M. Hajjar, K. D. Smith, C. B. Wilson, L. Schroeder, A. Aderem, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13766; b) M. A. van Zoelen, H. Yang, S. Florquin, J. C. Meijers, S. Akira, B. Arnold, P. P. Nawroth, A. Bierhaus, K. J. Tracey, T. van der Poll, *Shock* **2009**, *31*, 280;
c) T. Kawai, S. Akira, *Nat. Immunol.* **2010**, *11*, 373.
[14] A. D. Luster, R. Alon, U. H. von Andrian, *Nat. Immunol.* **2005**, *6*, 1182.
[15] E. Kolaczkowska, P. Kubes, *Nat. Rev. Immunol.* **2013**, *13*, 159.
[16] O. Soehnlein, L. Lindbom, *Nat. Rev. Immunol.* **2010**, *10*, 427.
[17] M. Horckmans, L. Ring, J. Duchene, D. Santovito, M. J. Schloss, M. Drechsler, C. Weber, O. Soehnlein, S. Steffens, *Eur. Heart J.* **2016**, *38*, 187.
[18] N. Gao, J. Yin, G. S. Yoon, Q.-S. Mi, X. Y. Fu-Shin, *Am. J. Pathol.* **2011**, *179*, 2243.
[19] Y. Liu, L. Wang, T. Kikuiri, K. Akiyama, C. Chen, X. Xu, R. Yang, W. Chen, S. Wang, S. Shi, *Nat. Med.* **2011**, *17*, 1594.
[20] T. J. Koh, L. A. DiPietro, *Exp. Rev. Mol. Med.* **2011**, *13*, e23.
[21] a) L. C. Davies, S. J. Jenkins, J. E. Allen, P. R. Taylor, *Nat. Immunol.* **2013**, *14*, 986; b) S. Gordon, P. R. Taylor, *Nat. Rev. Immunol.* **2005**, *5*, 953.
[22] G. C. Gurtner, S. Werner, Y. Barrandon, M. T. Longaker, *Nature* **2008**, *453*, 314.
[23] A. Mantovani, S. K. Biswas, M. R. Galdiero, A. Sica, M. Locati, *J. Pathol.* **2013**, *229*, 176.
[24] M. L. Novak, T. J. Koh, *J. Leukocyte Biol.* **2013**, *93*, 875.
[25] D. M. Mosser, J. P. Edwards, *Nat. Rev. Immunol.* **2008**, *8*, 958.
[26] A. D'Andrea, X. Ma, M. Aste-Amezaga, C. Paganin, G. Trinchieri, *J. Exp. Med.* **1995**, *181*, 537.
[27] M. Lutolf, J. Hubbell, *Nat. Biotechnol.* **2005**, *23*, 47.
[28] J. A. Hubbell, *Nat. Biotechnol.* **1995**, *13*, 565.
[29] N. D. Leipzig, M. S. Shoichet, *Biomaterials* **2009**, *30*, 6867.
[30] a) J.-h. Lee, Y. J. Lee, H.-j. Cho, H. Shin, *Tissue Eng., Part A* **2014**, *20*, 2031; b) Y. M. Shin, H. J. Shin, Y. Heo, I. Jun, Y.-W. Chung, K. Kim, Y. M. Lim, H. Jeon, H. Shin, *J. Mater. Chem. B* **2017**, *5*, 318;
c) S. H. Ku, C. B. Park, *Adv. Healthcare Mater.* **2013**, *2*, 1445.
[31] E. S. Place, N. D. Evans, M. M. Stevens, *Nat. Mater.* **2009**, *8*, 457.
[32] D. S. Benoit, M. P. Schwartz, A. R. Durney, K. S. Anseth, *Nat. Mater.* **2008**, *7*, 816.
[33] Y. Qian, L. Li, Y. Song, L. Dong, P. Chen, X. Li, K. Cai, O. Germershaus, L. Yang, Y. Fan, *Biomaterials* **2018**, *164*, 22.
[34] F. Y. McWhorter, T. Wang, P. Nguyen, T. Chung, W. F. Liu, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 17253.
[35] H. C. Bygd, K. D. Forsmark, K. M. Bratlie, *Biomaterials* **2015**, *56*, 187.
[36] J. M. Anderson, A. Rodriguez, D. T. Chang, *Semin. Immunol.* **2008**, *20*, 86.
[37] F. Taraballi, M. Sushnitha, C. Tsao, G. Bauza, C. Liverani, A. Shi, E. Tasciotti, *Adv. Healthcare Mater.* **2018**, *7*, 1800490.
[38] P. Roach, D. Farrar, C. C. Perry, *J. Am. Chem. Soc.* **2005**, *127*, 8168.
[39] C. J. Wilson, R. E. Clegg, D. I. Leavesley, M. J. Pearcy, *Tissue Eng.* **2005**, *11*, 1.
[40] W.-J. Hu, J. W. Eaton, T. P. Ugarova, L. Tang, *Blood* **2001**, *98*, 1231.
[41] K. Nakamura, S. Yokohama, M. Yoneda, S. Okamoto, Y. Tamaki, T. Ito, M. Okada, K. Aso, I. Makino, *J. Gastroenterol.* **2004**, *39*, 346.
[42] A. Kerschenmeyer, Ø. Arlov, V. Malheiro, M. Steinwachs, M. Rottmar, K. Maniura-Weber, G. Palazzolo, M. Zenobi-Wong, *Biomater. Sci.* **2017**, *5*, 1756.
[43] Y. Zhong, R. V. Bellamkonda, *Brain Res.* **2007**, *1148*, 15.
[44] a) D. Kwon, B. G. Cha, Y. Cho, J. Min, E.-B. Park, S.-J. Kang, J. Kim, *Nano Lett.* **2017**, *17*, 2747; b) H. Kang, H. J. Jung, S. K. Kim, D. S. H. Wong, S. Lin, G. Li, V. P. Dravid, L. Bian, *ACS Nano* **2018**, *12*, 5978.
[45] K. Garg, N. A. Pullen, C. A. Oskeritzian, J. J. Ryan, G. L. Bowlin, *Biomaterials* **2013**, *34*, 4439.
[46] M. Friedemann, L. Kalbitzer, S. Franz, S. Moeller, M. Schnabelrauch, J. C. Simon, T. Pompe, K. Franke, *Adv. Healthcare Mater.* **2017**, *6*, 1600967.
[47] R. Ion, S. Vizireanu, C. E. Stancu, C. Luculescu, A. Cimpean, G. Dinescu, *Mater. Sci. Eng., C* **2015**, *48*, 118.
[48] J. A. Jones, D. T. Chang, H. Meyerson, E. Colton, I. K. Kwon, T. Matsuda, J. M. Anderson, *J. Biomed. Mater. Res., Part A* **2007**, *83A*, 585.
[49] M. Bartneck, H. A. Keul, S. Singh, K. Czaja, J. Bornemann, M. Bockstaller, M. Moeller, G. Zwadlo-Klarwasser, J. Groll, *ACS Nano* **2010**, *4*, 3073.
[50] a) T. A. Einhorn, L. C. Gerstenfeld, *Nat. Rev. Rheumatol.* **2015**, *11*, 45; b) J. A. Gasser, M. Kneissel, in *Bone Toxicology* (Eds: S. Y. Smith, A. Varela, R. Samadfam), Springer, Cham, Switzerland **2017**, p. 27.
[51] a) G. L. Lin, K. D. Hankenson, *J. Cell. Biochem.* **2011**, *112*, 3491; b) X. Chen, L. Wang, K. Zhao, H. Wang, *Tissue Eng., Part B* **2018**, *24*, 215.
[52] S. M. Abdelmagid, M. F. Barbe, F. F. Safadi, *Life Sci.* **2015**, *123*, 25.
[53] K. M. Hotchkiss, G. B. Reddy, S. L. Hyzy, Z. Schwartz, B. D. Boyan, R. Olivares-Navarrete, *Acta Biomater.* **2016**, *31*, 425.
[54] S. Hamlet, S. Ivanovski, *Acta Biomater.* **2011**, *7*, 2345.
[55] K. L. Spiller, S. Nassiri, C. E. Witherel, R. R. Anfang, J. Ng, K. R. Nakazawa, T. Yu, G. Vunjak-Novakovic, *Biomaterials* **2015**, *37*, 194.
[56] G. Arango Duque, A. Descoteaux, *Front. Immunol.* **2014**, *5*, 491.
[57] R. S. Taichman, P. V. Hauschka, *Inflammation* **1992**, *16*, 587.
[58] Q. Zhang, B. Chen, F. Yan, J. Guo, X. Zhu, S. Ma, W. Yang, *Biomed. Res. Int.* **2014**, *2014*, 5.
[59] G. Chen, C. Deng, Y.-P. Li, *Int. J. Biol. Sci.* **2012**, *8*, 272.
[60] a) J. Vlacic-Zischke, S. M. Hamlet, T. Friis, M. S. Tonetti, S. Ivanovski, *Biomaterials* **2011**, *32*, 665; b) X. Dai, Y. Wei, X. Zhang, S. Meng, X. Mo, X. Liu, X. Deng, L. Zhang, X. Deng, *J. Nanomater.* **2015**, *2015*, 1; c) S. Hamlet, M. Alfarsi, R. George, S. Ivanovski, *Clin. Oral Implants Res.* **2012**, *23*, 584.
[61] W. Brodbeck, Y. Nakayama, T. Matsuda, E. Colton, N. Ziats, J. Anderson, *Cytokine* **2002**, *18*, 311.
[62] a) Z. Chen, X. Mao, L. Tan, T. Friis, C. Wu, R. Crawford, Y. Xiao, *Biomaterials* **2014**, *35*, 8553; b) Z. Jia, P. Xiu, P. Xiong, W. Zhou, Y. Cheng, S. Wei, Y. Zheng, T. Xi, H. Cai, Z. Liu, C. Wang, W. Zhang, Z. Li, *ACS Appl. Mater. Interfaces* **2016**, *8*, 28495;
c) M. Wang, F. Chen, J. Wang, X. Chen, J. Liang, X. Yang, X. Zhu, Y. Fan, X. Zhang, *J. Mater. Chem. B* **2018**, *6*, 4765.
[63] T. U. Luu, S. C. Gott, B. W. Woo, M. P. Rao, W. F. Liu, *ACS Appl. Mater. Interfaces* **2015**, *7*, 28665.
[64] E. M. Sussman, M. C. Halpin, J. Muster, R. T. Moon, B. D. Ratner, *Ann. Biomed. Eng.* **2014**, *42*, 1508.
[65] a) P. Guihard, Y. Danger, B. Brounais, E. David, R. Brion, J. Delecrin, C. D. Richards, S. Chevalier, F. Rédini, D. Heymann, *Stem Cells* **2012**, *30*, 762; b) F. Loi, L. A. Córdova, R. Zhang, J. Pajarinen, T.-h. Lin, S. B. Goodman, Z. Yao, *Stem Cell Res. Ther.* **2016**, *7*, 15; c) P. Luz-Crawford, F. Djouad, K. Toupet, C. Bony, M. Franquesa, M. J. Hoogduijn, C. Jorgensen, D. Noël, *Stem Cells* **2016**, *34*, 483.
[66] F. Wei, Y. Zhou, J. Wang, C. Liu, Y. Xiao, *Tissue Eng., Part A* **2018**, *24*, 584.

[67] X. Li, Y. Song, F. Liu, D. Liu, H. Miao, J. Ren, J. Xu, L. Ding, Y. Hu, Z. Wang, *J. Cell. Biochem.* **2017**, *118*, 2780.
[68] a) M. Wang, Y. Yu, K. Dai, Z. Ma, Y. Liu, J. Wang, C. Liu, *Biomater. Sci.* **2016**, *4*, 1574; b) C.-H. Lee, Y.-J. Kim, J.-H. Jang, J.-W. Park, *Nanotechnology* **2016**, *27*, 085101.
[69] Z. Chen, C. Wu, W. Gu, T. Klein, R. Crawford, Y. Xiao, *Biomaterials* **2014**, *35*, 1507.
[70] R. Morent, N. De Geyter, T. Desmet, P. Dubruel, C. Leys, *Plasma Processes Polym.* **2011**, *8*, 171.
[71] B. D. Boyan, T. W. Hummert, D. D. Dean, Z. Schwartz, *Biomaterials* **1996**, *17*, 137.
[72] K. Shin, T. Acri, S. Geary, A. K. Salem, *Tissue Eng., Part A* **2017**, *23*, 1169.
[73] T. P. Kunzler, C. Huwiler, T. Drobek, J. Vörös, N. D. Spencer, *Biomaterials* **2007**, *28*, 5000.
[74] Z. Chen, A. Bachhuka, S. Han, F. Wei, S. Lu, R. M. Visalakshan, K. Vasilev, Y. Xiao, *ACS Nano* **2017**, *11*, 4494.
[75] Q.-L. Ma, L.-Z. Zhao, R.-R. Liu, B.-Q. Jin, W. Song, Y. Wang, Y.-S. Zhang, L.-H. Chen, Y.-M. Zhang, *Biomaterials* **2014**, *35*, 9853.
[76] R. Sridharan, A. R. Cameron, D. J. Kelly, C. J. Kearney, F. J. O'Brien, *Mater. Today* **2015**, *18*, 313.
[77] M. Arnold, V. C. Hirschfeld-Warneken, T. Lohmüller, P. Heil, J. Blümmel, E. A. Cavalcanti-Adam, M. López-García, P. Walther, H. Kessler, B. Geiger, *Nano Lett.* **2008**, *8*, 2063.
[78] J. Leijten, A. Khademhosseini, *Cell Stem Cell* **2016**, *18*, 20.
[79] K. S. Brammer, S. Oh, C. J. Frandsen, S. Jin, in *Biomaterials Science and Engineering* (Ed: R. Pignatello), InTech, London **2011**.
[80] Z. Chen, S. Ni, S. Han, R. Crawford, S. Lu, F. Wei, J. Chang, C. Wu, Y. Xiao, *Nanoscale* **2017**, *9*, 706.
[81] D.-H. Kim, J. T. Smith, A. Chilkoti, W. M. Reichert, *Biomaterials* **2007**, *28*, 3369.
[82] S. D'Mello, K. Atluri, S. M. Geary, L. Hong, S. Elangovan, A. K. Salem, *AAPS J.* **2017**, *19*, 43.
[83] S. L. Teitelbaum, *Science* **2000**, *289*, 1504.
[84] H. C. Blair, S. L. Teitelbaum, R. Ghiselli, S. Gluck, *Science* **1989**, *245*, 855.
[85] M. Chellaiah, N. Kizer, M. Silva, U. Alvarez, D. Kwiatkowski, K. A. Hruska, *J. Cell Biol.* **2000**, *148*, 665.
[86] J. C. Crockett, M. J. Rogers, F. P. Coxon, L. J. Hocking, M. H. Helfrich, *J. Cell Sci.* **2011**, *124*, 991.
[87] D. J. Hadjidakis, I. I. Androulakis, *Women's Health Dis.: Gynecol. Endocr. Reprod. Issues* **2006**, *1092*, 385.
[88] M. C. Walsh, N. Kim, Y. Kadono, J. Rho, S. Y. Lee, J. Lorenzo, Y. Choi, *Annu. Rev. Immunol.* **2006**, *24*, 33.
[89] S. Wei, H. Kitaura, P. Zhou, F. P. Ross, S. L. Teitelbaum, *J. Clin. Invest.* **2005**, *115*, 282.
[90] a) M. C. Horowitz, J. A. Fretz, *J. Bone Miner. Res.* **2012**, *27*, 1448; b) M. C. Walsh, Y. Choi, *Front. Immunol.* **2014**, *5*, 511.
[91] P. Palmqvist, E. Persson, H. H. Conaway, U. H. Lerner, *J. Immunol.* **2002**, *169*, 3353.
[92] I. R. Reid, C. Lowe, J. Cornish, S. J. M. Skinner, D. J. Hilton, T. A. Willson, D. P. Gearing, T. J. Martin, *Endocrinology* **1990**, *126*, 1416.
[93] M. K. Park, Y. M. Her, M. La Cho, H. J. Oh, E. M. Park, S. K. Kwok, J. H. Ju, K. S. Park, D. S. Min, H. Y. Kim, S. H. Park, *Immunol. Lett.* **2011**, *139*, 42.
[94] M. S. Bendre, D. C. Montague, T. Peery, N. S. Akel, D. Gaddy, L. J. Suva, *Bone* **2003**, *33*, 28.
[95] F. Grassi, A. Piacentini, S. Cristino, S. Toneguzzi, C. Cavallo, A. Facchini, G. Lisignoli, *Histochem. Cell Biol.* **2003**, *120*, 391.
[96] K. Mizutani, S. Sud, N. A. McGregor, G. Martinovski, B. T. Rice, M. J. Craig, Z. S. Varsos, H. Roca, K. J. Pienta, *Neoplasia* **2009**, *11*, 1235.
[97] Y. Okamatsu, D. Kim, R. Battaglino, H. Sasaki, U. Spate, P. Stashenko, *J. Immunol.* **2004**, *173*, 2084.
[98] S. M. Bang, H. J. Moon, Y. D. Kwon, J. Y. Yoo, A. Pae, I. K. Kwon, *Clin. Oral Implants Res.* **2014**, *25*, 831.
[99] D. O. Costa, P. D. H. Prowse, T. Chrones, S. M. Sims, D. W. Hamilton, A. S. Rizkalla, S. J. Dixon, *Biomaterials* **2013**, *34*, 7215.
[100] L. Forte, P. Torricelli, E. Boanini, M. Gazzano, K. Rubini, M. Fini, A. Bigi, *Acta Biomater.* **2016**, *32*, 298.
[101] S. H. Lee, T. S. Kim, Y. Choi, J. Lorenzo, *BMB Rep.* **2008**, *41*, 495.
[102] Y. Funaki, Y. Hasegawa, R. Okazaki, A. Yamasaki, Y. Sueda, A. Yamamoto, M. Yanai, T. Fukushima, T. Harada, H. Makino, E. Shimizu, *Yonago Acta Med.* **2018**, *61*, 8.
[103] Y. Morita, H. Kitaura, M. Yoshimatsu, Y. Fujimura, H. Kohara, T. Eguchi, N. Yoshida, *Calcif. Tissue Int.* **2010**, *86*, 242.
[104] W. Zhang, X. L. Cong, Y. H. Qin, Z. W. He, D. Y. He, S. M. Dai, *Inflammation* **2013**, *36*, 103.
[105] M. Yoshimatsu, H. Kitaura, Y. Fujimura, T. Eguchi, H. Kohara, Y. Morita, N. Yoshida, *Bone* **2009**, *45*, 1010.
[106] M. Roy, S. Bose, *J. Biomed. Mater. Res., Part A* **2012**, *100A*, 2450. [107] C. Steffi, Z. Shi, C. H. Kong, W. Wang, *J. Funct. Biomater.* **2018**, *9*, 18.
[108] S. Yamada, D. Heymann, J. M. Bouler, G. Daculsi, *Biomaterials* **1997**, *18*, 1037.
[109] I. Wepener, W. Richter, D. van Papendorp, A. M. Joubert, *J. Mater. Sci.: Mater. Med.* **2012**, *23*, 3029.
[110] C. M. Botelho, R. A. Brooks, G. Spence, I. McFarlane, M. A. Lopes, S. M. Best, J. D. Santos, N. Rushton, W. Bonfield, *J. Biomed. Mater. Res., Part A* **2006**, *78A*, 709.
[111] N. L. Davison, J. Su, H. Yuan, J. J. J. P. van den Beucken, J. D. de Bruijn, F. B. D. Groot, *Eur. Cells Mater.* **2015**, *29*, 314.
[112] B. Sommer, R. Felix, C. Sprecher, M. Leunig, R. Ganz, W. Hofstetter, *J. Biomed. Mater. Res.* **2005**, *72A*, 67.
[113] J. Brinkmann, T. Hefti, F. Schlottig, N. D. Spencer, H. Hall, *Biointerphases* **2012**, *7*, 34.
[114] M. Shemesh, L. Addadi, B. Geiger, *J. R. Soc., Interface* **2017**, *14*, 20160958.
[115] C. Q. Yi, D. D. Liu, C. C. Fong, J. C. Zhang, M. S. Yang, *ACS Nano* **2010**, *4*, 6439.
[116] D. Lee, D. N. Heo, H. J. Kim, W. K. Ko, S. J. Lee, M. Heo, J. B. Bang, J. B. Lee, D. S. Hwang, S. H. Do, I. Kwon, *Sci. Rep.* **2016**, *6*, 27336.
[117] L. Forte, P. Torricelli, E. Boanini, K. Rubini, M. Fini, A. Bigi, *J. Biomed. Mater. Res., Part A* **2017**, *105*, 3293.
[118] A. K. Ghag, J. E. Gough, S. Downes, *Biomater. Sci.* **2014**, *2*, 233.
[119] F. Riccitiello, A. De Luise, R. Conte, S. D'Aniello, V. Vittoria, A. Di Salle, A. Calarco, G. Peluso, *Eur. Polym. J.* **2018**, *99*, 289.
[120] Z. T. Chen, T. Klein, R. Z. Murray, R. Crawford, J. Chang, C. T. Wu, Y. Xiao, *Mater. Today* **2016**, *19*, 304.
[121] a) R. Marsell, T. A. Einhorn, *Injury* **2011**, *42*, 551; b) A. D. Anastasilakis, P. Makras, M. P. Yavropoulou, G. T. Sakellariou, F. Papadopoulou, S. Gerou, S. E. Papapoulos, *Osteoporos Int.* **2017**, *28*, S156.
[122] a) J. W. Cheng, L. Zhou, Q. Liu, J. Tickner, Z. Tan, X. F. Li, M. Liu, X. X. Lin, T. Wang, N. J. Pavlos, J. M. Zhao, J. K. Xu, *J. Cell. Physiol.* **2018**, *233*, 2502; b) S. Chen, G. Jin, K. M. Huang, J. J. Ma, Q. Wang, Y. Ma, X. Z. Tang, Z. J. Zhou, Z. J. Hu, J. Y. Wang, A. Qin, S. W. Fan, *Sci. Rep.* **2015**, *5*, 12853.
[123] Y. Li, F. Li, C. C. Zhang, B. A. Gao, P. Tan, B. G. Mi, Y. Zhang, H. Cheng, H. Liao, K. F. Huo, W. Xiong, *J. Nanosci. Nanotechnol.* **2015**, *15*, 4136.
[124] a) F. R. Maia, S. J. Bidarra, P. L. Granja, C. C. Barrias, *Acta Biomater.* **2013**, *9*, 8773; b) H. F. Hildebrand, N. Blanchemain, G. Mayer, Y. M. Zhang, O. Melnyk, M. Morcellet, B. Martel, *Adv. Biomater. VI* **2005**, *288–289*, 47.
[125] X. F. Wang, B. Ding, B. Y. Li, *Mater. Today* **2013**, *16*, 229.
[126] a) J. J. Li, A. Akey, C. R. Dunstan, M. Vielreicher, O. Friedrich, D. C. Bell, H. Zreiqat, *Adv. Healthcare Mater.* **2018**, *7*, https://doi.org/10.1002/adhm.201800218; b) W. Orapiriyakul, P. S. Young, L.

Damiati, P. M. Tsimbouri, *J. Tissue Eng.* **2018**, *9*, https://doi.org/10.1177/2041731418789838.
[127] J. G. Souza, J. M. Cordeiro, C. V. Lima, V. A. Barão, *J. Periodontol.* **2018**.
[128] S. Arima, H. Ochi, M. Mitsuhashi, R. Kibe, K. Takahashi, Y. Kataoka, *Lett. Appl. Microbiol.* **2018**, *67*, 214.
[129] X. Li, G. S. Cheung, G. S. Watson, J. A. Watson, S. Lin, L. Schwarzkopf, D. W. Green, *Nanoscale* **2017**, *9*, 464.
[130] C. D. Bandara, S. Singh, I. O. Afara, A. Wolff, T. Tesfamichael, K. Ostrikov, A. Oloyede, *ACS Appl. Mater. Interfaces* **2017**, *9*, 6746.
[131] X. Xu, L. Wang, Z. Luo, Y. Ni, H. Sun, X. Gao, Y. Li, S. Zhang, Y. Li, S. Wei, *ACS Appl. Mater. Interfaces* **2017**, *9*, 43300.
[132] C. T. Johnson, J. A. Wroe, R. Agarwal, K. E. Martin, R. E. Guldberg, R. M. Donlan, L. F. Westblade, A. J. García, *Proc. Natl. Acad. Sci USA* **2018**, https://doi.org/10.1073/pnas.1801013115.
[133] H. C. Flemming, J. Wingender, *Nat. Rev. Microbiol.* **2010**, *8*, 623.
[134] a) J. A. Geoghegan, T. J. Foster, in *Staphylococcus aureus: Microbiology, Pathology, Immunology, Therapy and Prophylaxis* (Eds: F. Bagnoli, R. Rappuoli, G. Grandi), Springer, Cham, Switzerland **2017**, p. 95; b) T. Foster, *Eur. J. Clin. Microbiol. Infect. Dis.* **2016**, *35*, 1923.
[135] B. F. Ricciardi, G. Muthukrishnan, E. Masters, M. Ninomiya, C. C. Lee, E. M. Schwarz, *Curr. Rev. Musculoskeletal Med.* **2018**, *11*, 389.
[136] K. M. Rigby, F. R. DeLeo, *Semin. Immunopathol.* **2012**, *34*, 237.
[137] a) C. M. Gries, T. Kielian, *J. Am. Acad. Orthop. Surg.* **2017**, *25*, S20; b) T. D. Scherr, C. E. Heim, J. M. Morrison, T. Kielian, *Front. Immunol.* **2014**, *5*, 37.
[138] J.-Y. Kim, K.-H. Kim, E.-H. Kwag, Y. J. Seol, Y. M. Lee, Y. Ku, I.-C. Rhyu, *J. Periodontal Implant Sci.* **2018**, *48*, 70.
[139] S. Isefuku, C. J. Joyner, A. H. R. Simpson, *J. Orthop. Trauma* **2003**, *17*, 212.
[140] S. Pogodin, J. Hasan, V. A. Baulin, H. K. Webb, V. K. Truong, T. H. Phong Nguyen, V. Boshkovikj, C. J. Fluke, G. S. Watson, J. A. Watson, R. J. Crawford, E. P. Ivanova, *Biophys. J.* **2013**, *104*, 835.
[141] J. Hasan, K. Chatterjee, *Nanoscale* **2015**, *7*, 15568.
[142] X. Li, *Phys. Chem. Chem. Phys.* **2016**, *18*, 1311.
[143] a) A. Jaggessar, H. Shahali, A. Mathew, P. K. Yarlagadda, *J. Nanobiotechnol.* **2017**, *15*, 64; b) X. Li, G. S. Cheung, G. S. Watson, J. A. Watson, S. Lin, L. Schwarzkopf, D. W. Green, *Nanoscale* **2016**, *8*, 18860.
[144] H. Pan, Y. Xie, Z. Zhang, K. Li, D. Hu, X. Zheng, T. Tang, *Biomed. Mater.* **2017**, *12*, 045006.
[145] X. Xin, P. Li, Y. Zhu, L. Shi, J. Yuan, J. Shen, *Langmuir* **2018**, https://doi.org/10.1021/acs.langmuir.8b01603.
[146] H. Knopf-Marques, S. Singh, S. S. Htwe, L. Wolfova, R. Buffa, J. Bacharouche, G. Francius, J. C. Voegel, P. Schaaf, A. M. Ghaemmaghami, N. E. Vrana, P. Lavalle, *Biomacromolecules* **2016**, *17*, 2189.
[147] K. Duske, L. Jablonowski, I. Koban, R. Matthes, B. Holtfreter, A. Sckell, J. B. Nebe, T. von Woedtke, K. D. Weltmann, T. Kocher, *Biomaterials* **2015**, *52*, 327.
[148] C. Sengstock, M. Lopian, Y. Motemani, A. Borgmann, C. Khare, P. J. Buenconsejo, T. A. Schildhauer, A. Ludwig, M. Koller, *Nanotechnology* **2014**, *25*, 195101.
[149] S. M. Skovdal, N. P. Jørgensen, E. Petersen, S. Jensen-Fangel, R. Ogaki, G. Zeng, M. I. Johansen, M. Wang, H. Rohde, R. L. Meyer, *Acta Biomater.* **2018**, *76*, 46.
[150] R. Zhang, X. Liu, Z. Xiong, Q. Huang, X. Yang, H. Yan, J. Ma, Q. Feng, Z. Shen, *Artif. Cells, Nanomed., Biotechnol.* **2018**, https://doi.org/10.1080/21691401.2018.14464421.
[151] L. Bai, Y. Liu, Z. Du, Z. Weng, W. Yao, X. Zhang, X. Huang, X. Yao, R. Crawford, R. Hang, D. Huang, B. Tang, Y. Xiao, *Acta Biomater.* **2018**, *76*, 344.
[152] S. Kheur, N. Singh, D. Bodas, J. Y. Rauch, S. Jambhekar, M. Kheur, J. Rajwade, *Colloids Surf., B* **2017**, *159*, 151.
[153] M. Radunovic, M. De Colli, P. De Marco, C. Di Nisio, A. Fontana, A. Piattelli, A. Cataldi, S. Zara, *J. Biomed. Mater. Res., Part A* **2017**, *105*, 2312.
[154] H. Li, D. Tang, C. Qi, X. Zhao, G. Wang, Y. Zhang, T. Yu, *J. Orthop. Surg. Res.* **2018**, *13*, 139.
[155] H. Cheng, K. Yue, M. Kazemzadeh-Narbat, Y. Liu, A. Khalilpour, B. Li, Y. S. Zhang, N. Annabi, A. Khademhosseini, *ACS Appl. Mater. Interfaces* **2017**, *9*, 11428.
[156] J. D. Glaeser, K. Salehi, L. E. A. Kanim, D. Sheyn, Z. NaPier, P. H. Behrens, L. Garcia, J. M. Cuellar, H. W. Bae, *Tissue Eng., Part A* **2018**, https://doi.org/10.1089/ten.tea.2017.0512.
[157] H. Geng, Y. Yuan, A. Adayi, X. Zhang, X. Song, L. Gong, X. Zhang, P. Gao, *Mater. Sci. Eng., C* **2018**, *82*, 141.
[158] M. Hoyos-Nogues, F. Velasco, M. P. Ginebra, J. M. Manero, F. J. Gil, C. Mas-Moruno, *ACS Appl. Mater. Interfaces* **2017**, *9*, 21618.
[159] H. Yazici, M. B. O'Neill, T. Kacar, B. R. Wilson, E. E. Oren, M. Sarikaya, C. Tamerler, *ACS Appl. Mater. Interfaces* **2016**, *8*, 5070.
[160] S. E. Kim, S. H. Song, Y. P. Yun, B. J. Choi, I. K. Kwon, M. S. Bae, H. J. Moon, Y. D. Kwon, *Biomaterials* **2011**, *32*, 366.
[161] M. A. Alfarsi, S. M. Hamlet, S. Ivanovski, *Clin. Implant Dent. Relat. Res.* **2015**, *17*, 1036.

牙科鈦植體表面製備

國家科學及技術委員會補助專題研究計畫報告 牙科鈦植體表面製備仿細胞外基質特性之三維結構以促進骨組 織之反應(第 3 年) 報告類別：成果報告 計畫類別：個別型計畫 計畫編號：MOST 108-2314-B-010-013-MY3 執行期間：110 年 08 月 01 日至 111 年 07 月 31 日 執行單位：國立陽明大學牙醫學系 計畫主持人：黃何雄 共同主持人：孫瑛穗 計畫參與人員：碩士級-專任助理：鄭郁穎碩士班研究生-兼任助理：陸玟儒 碩士班研究生-兼任助理：葉建良 碩士班研究生-兼任助理：陳定澤 碩士班研究生-兼任助理：沈峻育 博士班研究生-兼任助理：温家儀 博士後研究-博士後研究：劉珈妃 報告附件：出席國際學術會議心得報告 本研究具有政策應用參考價值：■否 □是，建議提供機關 （勾選

「是」者，請列舉建議可提供施政參考之業務主管機關）

本研究具影響公共利益之重大發現：□否 □是 中 華 民 國 111 年 11 月 16 日 中文摘要：噴砂/酸蝕為目前牙科鈦植體常用的表面處理方式。然而利用此表面處理方式製成之鈦植體仍屬於生物惰性。因此，本計畫提出以三年 時間開發新一代牙科植體表面處理技術，牙科植體表面製備仿細胞 外基質之三維纖維網結構及其生物分子特性之表面，藉此主動誘導 並促進血管新生及成骨反應。本計畫前期透過結合噴砂、酸蝕及鹼 蝕處理方式，於鈦表面製備出不具潛在細胞毒性、但擁有良好親水 性及生物活性，厚度約數百奈米之三維纖維網狀孔洞結構表面，達 到仿細胞外基質結構形貌特性之目的；接續利用天然交聯劑原花

青素交聯第一型膠原蛋白接枝處理，成功將第一型膠原蛋白接枝於前 述三維纖維網狀表面，達到三維結構及主要生物分子種類皆仿細胞 外基質特性之目標；並進一步以小鼠巨噬細胞 (RAW 264.7)、人類 臍靜脈內皮細胞 (human umbilical vein endothelial cells, HUVECs)及人類間葉骨髓幹細胞 (human bone marrow mesenchymal stem cells, hMSCs)分析表面處理前後對巨噬細胞極化、血管新生及成骨作用之影響。研究結果顯示，本計畫製備之仿細胞外基質三 維纖維網狀結構表面可促使巨噬細胞極化為 M2 亞型，並進一步對血 管新生及成骨分化均產生正向影響；另一方面，在仿細胞外基質三 維形貌結構上透過原花青素交聯膠原蛋白接枝處理組別可提供適合 HUVECs 及 hMSCs 細胞貼附及增生之環境，亦可直接促進 HUVECs 管柱 形成及 hMSCs 胞外基質礦化等血管新生與成骨分化表現。本研究計畫 所開發仿細胞外基質特性之鈦金屬表面處理技術具有應用於牙科鈦植體表面處理之發展潛力，未來將透過動物植入試驗進一步驗證其骨整合能力。 中文關鍵詞：牙科鈦植體、表面處理、噴砂/酸蝕/鹼蝕處理、仿細胞外基質、原 花青素、第一型膠原蛋白、三維纖維網狀結構、巨噬細胞極化、血 管新生、成骨反應。 英文摘要：Sand-blasting/acid-etching (SLA) process is a gold standard for titanium (Ti) dental implant surface modification. However,

[142] SLA-modified Ti surface still shows bioinert property which can't spontaneously initiate cell responses after implantation. Therefore, the aim of this study was to develop a complex surface modification, i.e. combining SLA, alkaline treatment, and type I collagen immobilization using procyanidin cross-linking, to mimic the 3D porous structure and biomolecule property of extracellular matrix (ECM) for enhancing the angiogenesis and osteogenesis during bone healing. The results showed that a hydrophilic ECM-mimic 3D fibrous network structure showing well bioactivity was fabricated on Ti surfaces by using complex sand-blasting/acidetching/alkaline treatment. Moreover, type I collagen was successfully immobilized on the ECM mimic 3D fibrous network structure through procyanidin cross-linking. This ECM-mimic characteristics could regulate macrophage polarization, then affect the angiogenesis and osteogenesis cell responses. The proposed ECM-mimic surface stimulated the polarization of RAW 264.7 mouse macrophage cells to the M2 subtype. The angiogenic cell responses demonstrated that this ECM-mimic 3D fibrous network surface immobilized with type I collagen made human umbilical vein endothelial cells (HUVECs) show well cell adhesion and proliferation, and improved tube formation ability of HUVECs. Moreover, this ECM-mimic surface environment improved human bone marrow mesenchymal stem cells (hMSCs) adhesion, ECM mineralization, and osteogenic differentiation. We concluded that the proposed ECM-mimic surface characteristics showed high potential for Ti dental implant application. Further in vivo study is needed to confirm the effect of this ECM-mimic surface characteristics on osseointegration. 英文關鍵詞：titanium dental implant, surface modification, sand blasting/acid-etching/alkaline treatment, extracellular matrix (ECM)-mimic, procyanidin, type I collagen, three dimensional (3D) fibrous network structure, macrophage polarization, angiogenesis, osteogenesis.