Combating Implant Infections: Shifting Focus from Bacteria to Host

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The widespread use of biomaterials to support or replace body parts is increasingly threatened by the risk of implant-associated infections. In the quest for finding novel anti-infective biomaterials, there generally has been a one-sided focus on biomaterials with direct antibacterial properties, which leads to excessive use of antibacterial agents, compromised host responses, and unpredictable effectiveness in vivo. This review sheds light on how host immunomodulation, rather than only targeting bacteria, can endow biomaterials with improved anti-infective properties. How antibacterial surface treatments are at risk to be undermined by biomaterial features that dysregulate the protection normally provided by critical immune cell subsets, namely, neutrophils and macrophages, is discussed. Accordingly, how the precise modification of biomaterial surface biophysical cues, or the incorporation of immunomodulatory drug delivery systems, can render biomaterials with the necessary immune-compatible and immune-protective properties to potentiate the host defense mechanisms is reviewed. Within this context, the protective role of host defense peptides, metallic particles, quorum sensing inhibitors, and therapeutic adjuvants is discussed. The highlighted immunomodulatory strategies may lay a foundation to develop anti-infective biomaterials, while mitigating the increasing threat of antibacterial drug resistance.

1. Introduction

The demand for orthopedic implants that enable rehabilitation of skeletal loss or function is growing worldwide due to an ageing population.[1,2] Despite the bioinertness of metallic implants and their presumed biocompatibility, their implantation comes with the risk of bacterial colonization and subsequent progression into implant-associated infection (IAI). The commensal skin bacteria, *Staphylococci*, and in particular *Staphylococcus aureus*, have a strong tendency to colonize foreign bodies and cause IAI.[3,4] Important for the underlying pathophysiology, *Staphylococci* are highly competent at producing biofilms on implant surfaces, which encapsulate the bacterial niche from the outside environment,[5,6] thereby protecting the bacteria from host defense systems and antibiotics.[7] Antibiotics are administered as a routine procedure.[8] However, as a consequence of widespread antibiotics usage, bacteria are exposed to sub-inhibitory concentrations of antibiotics at a larger scale, driving their development toward antibiotic resistance,[9] as illustrated by the emergence of methicillin-resistant *S. aureus* (MRSA).[10,11]

As an improvement over current clinically applied local antibiotics delivery systems,[12] the recent developments in implant surface engineering approaches allow better antimicrobial functionalities to be incorporated to allow for more tunable and controlled drug release.[13–15] The "race to the surface" model is popular among biomaterials researchers to predict the biomaterials fate, resulting from the competition between eukaryotic cells and bacteria at the material surface.[16] Using this template, implant antibacterial properties are being stemmed from direct contact killing mechanisms due to implant surface modifications[17,18] or firm immobilization of drugs,[19,20] as they both "shield" the implant from bacterial adhesion. The current anti-infective biomaterials strategies being explored can be categorized as: 1) implant functionalization with antibiotics or antibacterial drugs such as host defense peptides (HDPs), inorganic materials (e.g., chitosan and derivatives), and inorganics (e.g., silver, copper, and zinc metal nanoparticles (NPs)), 2) anti-biofilm surface modification (e.g., coating with antifouling polymers or quorum sensor inhibitors), or 3) physical surface changes for direct contact-killing properties (e.g., nanotubes, nanopillars, and metal implantation).[15,21] Emerging as a serious alternative or adjuvant therapy to antibiotics, bacteriophage (phage) therapy uses viruses responsible for the lysis of specific bacterial strains.[22,23] As a natural predator of bacteria, phages employ different killing mechanisms, making multidrug resistant bacteria susceptible for phages.[24] Moreover, phages are highly specific for pathogenic cells, which can eliminate disastrous off-target effects in the host.[25] As a limitation, the use of phage cocktails is needed for therapeutic efficacy,[26] while there is currently is no conclusive data on possible long-term side effects of phage therapy in

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humans.[25] Therefore, novel phage-functionalized materials are unlikely to reach clinical practice in the near future.

As a key aspect in the pathogenesis of IAI, the inherent immunomodulating role of the biomaterial and its interplay with the host's innate immunity is often disregarded. As a result, the dysregulation of the hosts' defense by non-immunocompatible biomaterials can overrule their direct antibacterial functionalities, or even aggravate bacterial spread. The fate of an orthopedic implant is largely determined by its effects on the host immune response, as the anti-biofilm and tissue-supporting properties are processes that are both highly dependent on the local immune status.[27,28] In general, persisting inflammation impedes tissue repair and favors bacterial overgrowth.[28–32] A balanced inflammatory environment around the biomaterial is critical, since both downregulated and excessive inflammatory responses lead to suboptimal bone regeneration clinically.[32,33] Moreover, a balanced inflammation seems to be an optimal state at which the host innate immune system operates to eradicate infections. On the one hand, absence of inflammation is deleterious to the host as it is incapable of eradicating bacterial infection.[34,35] Once established, bacterial biofilms can leverage this weakness by attenuating the host's inflammatory response to resist clearance.[36] On the other hand, sustained inflammation is also associated with an altered susceptibility to infection, as can be seen in proinflammatory wear particle disease.[28,37,38] Likewise, the immune frustration resulting from the implantation of a non-immunocompatible biomaterial, leads to reduced bacterial recognition and phagocytosis, and hence, inefficient clearance of free-floating bacteria by the host.[39] As a result, a significantly reduced number of bacteria are needed to cause an infection.[28]

The primary goal of impactful immunomodulatory strategies should be the prevention of IAI, which is the focus of the current review. This requires efficient targeting by the biomaterial of pathogenic bacteria in their free-floating form, that is, when bacterial colonization can still be prevented and when they are most susceptible to direct eradication by host cellular immunity (i.e., comprising predominantly neutrophils and macrophages). Taking into consideration the main causative pathogens in IAI, the innate arm of immunity is known to be more efficient in eradicating *Staphylococcal* species as compared to the antigen-specific response mediated by the adaptive arm of immunity (i.e., dendritic cells (DCs) and T and B cells). In addition, once infection reaches the biofilm stage, biofilm-residing bacteria employ various strategies to hijack the host immune response, necessitating alternative immunological approaches dedicated to biofilm treatment, i.e., mostly involving immune reactivation strategies as reviewed by Seebach and Kubatzky.[39] As criteria for preventive anti-infective biomaterials, they should be immune-compatible, i.e., providing a minimal inflammatory response, while being nontoxic and supporting host bacterial killing. Second, implants with the appropriate immunomodulatory properties can further encourage host immunity to eradicate bacterial challenges (immune-protective properties).

Here, we review the key considerations in the development of immunomodulatory anti-infective biomaterials in light of the increasing threat of antibiotic resistance. The mechanisms are discussed by which biomaterials can cause defective local immune responses, predisposing them toward IAI. Specifically, the roles of neutrophils and macrophages in the host defense and inflammatory responses are highlighted, as the fate of the biomaterial is thought to be largely dependent on the initial reaction of these crucial innate immune cell players.[40–43] Based on the premise that appropriate immune-protection by the host is key, surface properties can be precisely modified to influence the biomaterial–host interactions, for which the effect of surface biophysical cues can be used as an example. Moreover, strategies for biomaterial surface functionalization with systems for delivery of immunomodulatory therapeutics—including host defense peptides, metallic nanoparticles, quorum sensing (QS) inhibitors, and therapeutic adjuvants—are discussed. These classes of agents are discussed in detail, as they have an immunological mode-of-action that can specifically target the early stages of infection. Last, suggestions are given for future avenues within this field, including novel multifunctional surface engineering approaches and methods for improved preclinical evaluation of immunomodulatory biomaterials.

2. Cellular Targets in Anti-Infective Biomaterial Development

2.1. Host Immune Response to Biomaterial Infection

S. aureus and *Staphylococcus epidermidis* are most often responsible for IAI, with the formation of a highly organized multicellular biofilm being a hallmark of the disease. The eradication of a mature biofilm is often extremely challenging, as the biofilm protects the bacteria from the immune system and antibiotics, often requiring surgical implant removal and long-term antibiotic treatment to overcome infection.[44] This tolerance of *Staphylococcal* biofilms is caused by the impermeability of the extracellular polymeric substances of the biofilm,[45] and the development of metabolically inactive, antibiotic-tolerant persister bacteria.[46] Furthermore, the biofilm consumes much of the environmental nutrients, leading to a further impaired immune response.[47]

Host immune responses that are effective in containment and clearance of *Staphylococcal* infections manifested by one growth type might not be effective against another growth type. Using several *S. aureus* and *S. epidermidis* isolates to monitor biofilm formation, it was shown that 80% of the isolates are biofilm-positive already within 3 days.[48] Accordingly, the prevention of bacterial adhesion onto the implant and their progression into a biofilm-residing phenotype during these critical first days should be the primary focus of immunomodulatory strategies, since the host immune system is considerably more effective in clearing planktonic, free-floating bacteria as compared to biofilm-type bacteria seen during chronic infection.[49] Several lines of evidence pinpoint innate immune cells as critical cell targets in anti-infective biomaterials strategies. Several innate immune cells already attach to biomaterials within a timeframe of hours, while lymphocytes are not initially observed.[29,41] Moreover, neutrophils and macrophages are the main innate immune effectors against planktonic *Staphylococci* species.[50] Their ability to directly kill bacteria is a prerequisite for successful infection clearance. The depletion of neutrophils or macrophages leads to the inability of the host to remove the bacterial burden, and is often associated with mortality.[51–55]

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Macrophages and neutrophils also orchestrate the balanced inflammation and tissue healing response needed for host integration of the biomaterial.[29] As highlighted in the current review, biomaterials can induce a "frustrated" state of these cells, leading to significantly reduced bacterial killing and tissue healing properties.

Adaptive immunity, comprising cellular and humoral immunity provided by T and B cells, has the ability to mount pathogen-specific, long-lasting protection to recurrent infections. Nevertheless, various studies using mice lacking functional T or B cells have indicated that the adaptive immune arm is not essential for initial clearance of *Staphylococci*.[55,56] In addition, no vaccination strategies have been able to mount T cell-mediated *Staphylococcal* immunity that protects against disease.[57] The role of adaptive immune cells in implant osseointegration is also debated, and might involve regulation of bone mineralization and remodeling.[58,59] Accordingly, only specialized T cell subsets were found to be involved in early bone regeneration.[60,61]

Although there are contradicting data about the presence and activation status of T cells around infected bone implants (recently reviewed by Seebach and Kubatzky[39]), T cells can prevail once the chronic biofilm stage of IAI is reached. T cells become activated in chronically infected bone tissue, but immune suppression exerted by biofilms seem to alter the normal T cell immunity. This is illustrated by a decreased proliferative capacity of T cells,[62] downregulating of T cell homing to the site of infection,[49,63] or mounting of T helper (Th)-17 responses that are unable to clear infection.[64,65] Their suppression by the biofilm environment may explain why only few T cells are sometimes found around infected prostheses.[62,66] The reactivation of T cell immunity to treat mature biofilms, e.g., by means of immune checkpoint inhibitors or activating specific T cell subsets, is beyond the scope of this review, and has been reviewed elsewhere.[39] Similarly, modulation of DC activity may be appropriate for the treatment of mature biofilms, as dendritic cells are activated by *Staphylococci* and have a key role in activating adaptive immunity.[67] Recently, DC-targeting biomaterial approaches to mediate T cell polarization toward Th1- or Th2-dominated responses have received attention. Although chemical coating or surface modifications can indeed modulate DC maturation,[68,69] biomaterial-regulation of DCs has not yet shown effectiveness in altering T cell responses to *Staphylococci*.[39,70,71]

2.2. Neutrophils—First Responders around the Biomaterial

The tissue damage resulting from biomaterial implantation leads to immediate activation of the coagulation cascade and the subsequent priming of innate immunity.[29] Moreover, the release of alarmins such as high mobility group box 1 (HMGB1), S100s, and heat-shock proteins from injured cells engage pathogen-recognition receptors (PRRs) to propagate inflammation and wound repair.[72] As the primary immune-surveillance arm of the innate immune system, neutrophils are activated within minutes and are the first cell type to congregate around a biomaterial,[41,73] where they are responsible for the clearance of cellular debris and pathogens by means of phagocytosis, reactive oxygen species (ROS) production, degranulation, and the

generation of pathogen-encapsulating neutrophil extracellular traps (NETs).[74–76] In addition, neutrophils produce an array of cytokines (i.e., interleukin (IL)-1β, IL-6, and IL-10) and chemokines (i.e., MCP-1 and CXCL1) to attract monocytes and further propagate the inflammatory response.[42] Neutrophils play a prominent role in fighting infection around the implant and, in particular, those originating from *Staphylococci*.[77,78] The intracellular granules of neutrophils contain numerous potent antimicrobial proteins and components for generating high levels of ROS, rendering them extremely competent in the intracellular killing of bacteria.[79] The observations that a decreased neutrophil function around the implant significantly increases the risk of biomaterial infection,[80,81] stresses the importance of having a normal neutrophil function around the biomaterial.

Several biomaterial-specific events lead to additional activation of neutrophils compared to normal wound healing. First, coverage of the biomaterial with extracellular matrix/blood proteins and complement factors forms additional binding sites for the adhesion and activation of neutrophils in the host immune response.[27,82] Second, increasing data show that the normal function of neutrophils is influenced by inherent biomaterial surface features.[43,83,84] Under ideal noninfected conditions, the presence of neutrophils around a biomaterial is confined to only a few days, as they should be quickly replaced by cells capable of dampening inflammation and initiating tissue regeneration.[85] When the neutrophil replacement mechanism fails, the sustained arsenal of antimicrobial effectors employed by neutrophils can be the cause of an undesired local immune milieu.[86] Thus, the right dynamics in neutrophil activation and not their sole presence is the key determining factor in a balanced inflammatory response.[87] From a bone healing perspective, persisting neutrophils and the accompanying inflammation will dysregulate the bone matrix production by delaying the recruitment of proangiogenic and pro-osteogenic macrophages,[33] or directly interfering with the differentiation of bone progenitor cells.[42,87,88] Excessive inflammation mediated by neutrophils can for instance be seen in chronic bone infections, major trauma or in the case of a non-immunocompatible biomaterial,[28,29,87] and are associated with a suppressed antibacterial function.[28] This local imbalance in host response is not easily reversed, as a defect in the first wave of neutrophils at the biomaterial will also lead to defective host defense mechanisms in subsequent waves of neutrophils.[89,90]

Neutrophils should be considered an important cell target in biomaterial-based immunomodulation, as biomaterial-mediated defects in their anti-infective mechanisms come at the risk of higher susceptibility to bacterial colonization and sustained inflammation[28,91] (**Figure 1**). At the same time, an exaggerated proinflammatory phenotype in neutrophils will have disastrous effects on the immunomodulatory activity of incoming macrophages and the bone formation by osteogenic cells.[83,87,88,90,92] Although the underlying mechanisms have not been clearly pinpointed to date, the defects observed in neutrophils in the vicinity of biomaterials involve either implant-induced metabolic exhaustion,[31] deactivation by host defense peptides,[93] excessive production of oxygen radicals,[31,84] and inflammasome activation,[94,95] all possibly leading to a deficient bacterial uptake and killing.[89,96,97] Uniquely, neutrophils also release
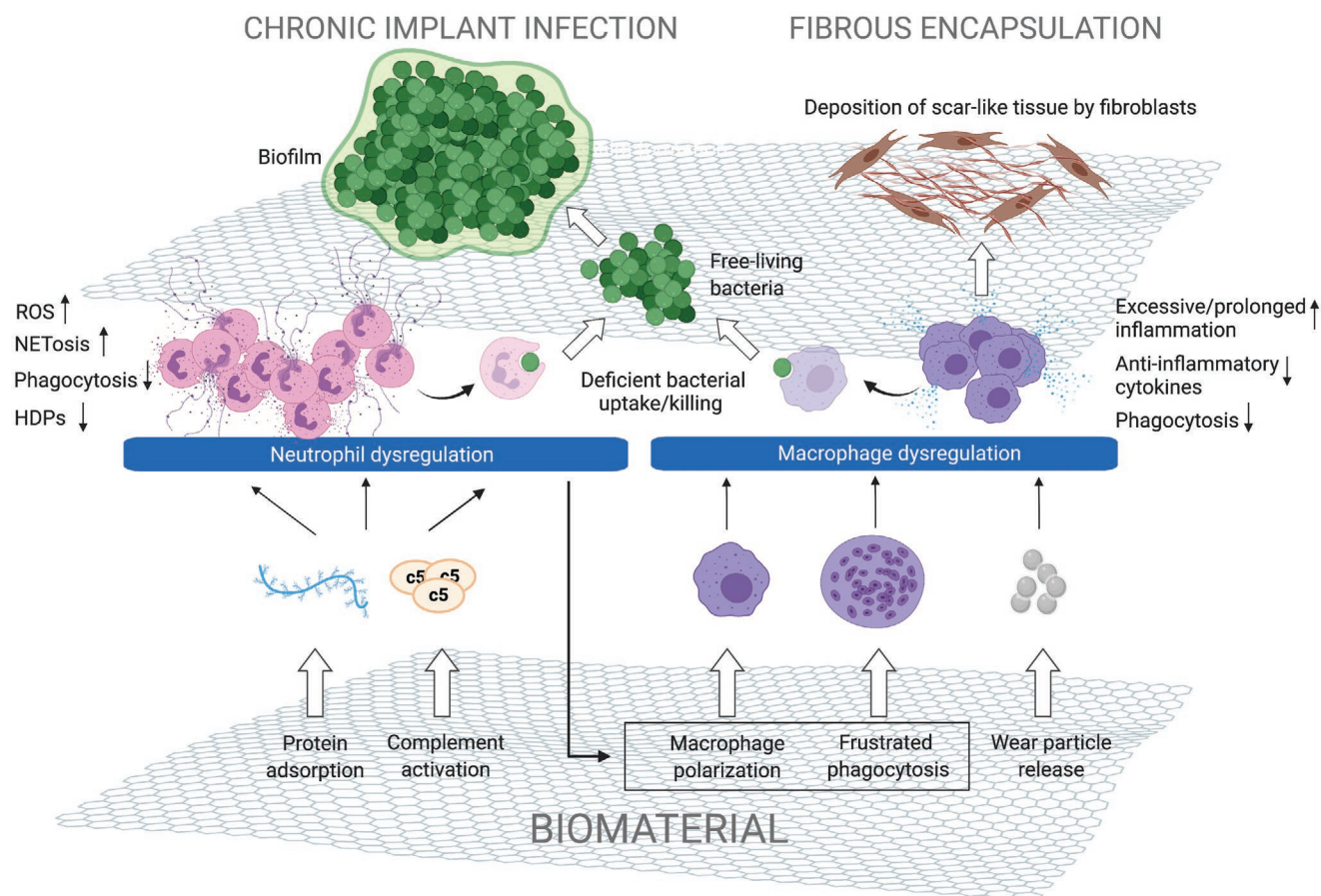
Figure 1. Biomaterial-mediated defects in the anti-infective host defense and healing responses. Left: The adsorption of blood/extracellular matrix proteins and complement factors to the biomaterial leads to the rapid activation of neutrophils. Neutrophils produce reactive oxygen species (ROS), degrading enzymes and undergo NETosis in response to the biomaterial and local trauma- or pathogen-related proinflammatory stimuli. Biomaterial-associated neutrophils are characterized by a deficient bacterial uptake and killing capacity, resulting from metabolic exhaustion, deactivation by HDPs, excessive ROS production and NETosis, and/or inflammasome activation. Following the acute phase, neutrophils produce various cytokines to recruit inflammatory macrophages. The defect in the first wave of neutrophils at the biomaterial is thought to contribute to the impaired host defense response of subsequent waves of neutrophils and macrophages. Right: In the presence of a persisting biomaterial, macrophages turn into a chronic inflammatory, "frustrated" state, characterized by the production of resorbing enzymes. Moreover, a decrease in bacterial-targeting activity reduces phagocytosis and alters the secretion of proinflammatory factors, shifting away from the classically activated macrophage phenotype. Additionally, their fusion into foreign body giant cells drives fibrous encapsulation of the biomaterial and prevents biomaterial–tissue integration.

NETs to extracellularly trap and kill bacteria, which is the last step in an active neutrophil death termed NETosis.[98] NETosis is exaggerated in the presence of a biomaterial, and is thought to be a main cause of the destructive inflammation around non-immunocompatible biomaterials, resulting in impaired neutrophil phagocytic ability and tissue healing[41,43,91,99–101] (Figures 1 and 2B). To create a host environment that eradicates bacteria and provides optimal wound healing, anti-infective strategies should aim at attenuating the neutrophil-mediated inflammation driven by uncontrolled ROS and NET production, while restoring or enhancing their anti-infective functions.

2.3. Macrophages—Diverse Immunomodulatory Players

As professional phagocytes, macrophages form a second line of defense against possible bacterial challenges around the biomaterial.[102] Furthermore, as a premise of their broad immunomodulatory functions, they strongly contribute to timely suppression of inflammation, revascularization, and tissue regeneration.[103,104] Pertinent to their role around orthopedic implants, macrophages produce an array of cytokines and growth factors that closely regulate the osteogenic behavior of mesenchymal stem cells (MSCs).[33] To further stress their importance around the biomaterial, macrophages will take a permanent place at the biomaterial–tissue interface. The materials immunocompatibility toward macrophages determines whether the biomaterial integrates with the body, or whether it is shielded from the body through fibrous encapsulation.[85]

Inflammatory macrophages originating from blood monocytes are recruited after biomaterial implantation, and subsequently undergo several phenotypic changes in adaptation to the local microenvironmental cues.[104] On each end of the spectrum, the major macrophage subtypes have been coined as either the M1 or M2 macrophage subtypes.[105] M1 macrophages are classically activated by strong inflammatory stimuli like

toll-like receptor (TLR) ligands or interferon-γ (IFN-γ), and responsible for proinflammatory cytokine production, phagocytosis and antigen presentation.[106] M2 macrophages arise after stimulation with IL-4, IL-13, and IL-10 and are primarily responsible for dampening the inflammatory response and orchestrating tissue regeneration.[107] Using this macrophage classification system, it is possible to summarize the prototypical response of macrophages to bacterial challenge with a proinflammatory M1 signature, as reviewed by Benoit et al.[108] Several effectors support the enhanced microbicidal activity of M1 macrophages and involve, for a large part, the capturing of bacteria within degradative phagolysosome, a process for which ROS and nitric oxide (NO) production are a prerequisite.[108,109] In comparison, M2 macrophages are more capable of removing foreign body particles or apoptotic/necrotic cells, consistent with their prohomeostatic role.[82] Furthermore, several genes related to M1 polarization are upregulated in response to bacterial infections, e.g., genes encoding the cytokines tumor necrosis factor (TNF)-α, IL-6, IL-12, IL-1β, and the chemokines such as CCL2, CCL5, and CXCL8.[108] Finally, a feature of the M1 macrophage is their increased ability to instruct adaptive immunity by antigen presentation.[110] Since the aforementioned features are all less profound in M2 macrophages, they are less capable of bacterial killing than M1 macrophages.[38,85] Both individual macrophage phenotypes are known to promote osteogenic differentiation in vitro, but it appears that sequential activation of M1 and M2 macrophages, respectively, leads to optimal bone formation.[33]

As presented in Figure 1, several events related to biomaterial implantation can cause macrophages to deviate from their characteristic microbicidal signature, leading to a higher susceptibility to IAI. First, macrophages are the driving force of a foreign body response around an implant, due to the inability of single macrophages to phagocytose and clear particles with sizes exceeding their own.[111] This causes macrophages to change into a "frustrated" state and fuse into foreign body giant cells (FBGCs).[27,85] The attempt of FBGCs to clear a nondegradable biomaterial is accompanied by a shift in phenotype reminiscent of neither classically nor alternatively activated macrophages, displaying a mixed production of anti- and proinflammatory cytokines, degrading enzymes and ROS, and with a decreased ability to phagocytose bacteria.[36] FBGCs are the driving force for the formation of a thick fibrous layer encapsulating the biomaterial, which hampers the long-term biomaterial–host integration as another major cause of biomaterial failure.[27,85] Second, once bacterial infection reaches the chronic stage, it utilizes different strategies to interfere with M1 polarization or promote M2 polarization, ultimately downgrading their microbicidal mechanisms.[39,108,112,113] The proposed changes underlying the polarization shift of macrophages toward anti-inflammatory M2 phenotype are a shift in their metabolism,[47] the production of anti-inflammatory cytokines including IL-10 and IL-12,[39,113] and the attenuation of MyD88/NF-κB activity.[7]

Surprisingly little is known about the feasibility of macrophage immunomodulation in favor of anti-infective biomaterials.[33,104,114] The induction of classically activated M1 macrophages can be identified as a potential strategy to restore the local host response to optimally fight a bacterial challenge.

An enhanced anti-infection response should, however, not come at the cost of an excessive or prolonged M1 macrophage activity, as this can lead to deleterious effects such as delayed angiogenesis,[103] impaired bone formation,[29,33] or uncontrolled osteoclast-mediated bone resorption.[9] On the one hand, the initial induction in prophagocytic macrophages could be followed by cues to promote the resolution of inflammation and attract the competent cells for angiogenesis and osteogenesis.[33,103] On the other hand, the M1/M2 macrophage classification system may be an overly simplified representation of the macrophages that are found in the continuum between the M1 and M2 extremes in vivo.[115] Accordingly, there is evidence to support that certain intermediate M1–M2 macrophage subsets may have specialized immunomodulatory and phagocytic functions that could help in the prevention of IAI.[116]

2.4. Host Immune Modulation by Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs are a population of immature myeloid cells, of which two major subsets can be identified based on cell surface marker expression and cell morphology, either resembling monocytes (M-MDSC) or granulocytes (G-MDSC).[117] As a distinct feature from other myeloid cells, MDSCs mainly exert strong immunosuppressive effects, manifesting particularly in pathologic conditions such as cancer, infection, and trauma.[66,118,119]

The role of MDSCs in biomaterial infections has only been established in recent years. Granulocytic MDSCs were found to be predominant leukocytes isolated from human prosthetic joint infections.[66] Moreover, *S. aureus* implant infection models have shown that MDSC infiltration is associated with reduced monocyte/macrophage counts.[120] Accordingly, the depletion of MDSCs restores the proinflammatory activity of monocytes/macrophages and leads to improved clearance of infection, showing that MDSCs actively contribute to a biofilm permissive environment.[120] The secretion of IL-10 by MDSCs, which is thought to enhance M2 macrophage polarization,[113] was pinpointed to underlie at least in part, the anti-inflammatory effects seen.[121] Altogether, these studies show that the recruitment and polarization of MDSCs is one of the mechanisms by which *S. aureus* biofilms skew the local immune response to an anti-inflammatory type and dampen the host defense system. Following this line of reasoning, it is likely that the influx of activated MDSCs may further exaggerate the immune-compromised environment that already exists around the biomaterial.

It is currently unknown if MDSCs can be successfully targeted to prevent IAI. As such, the functions of MSDCs during the onset of infection and various stages of biofilm formation is unclear. Moreover, since MDSCs regulate the polarization of macrophages, the influence of MDSCs on the bacterial killing phenotype of myeloid cells warrants investigation. A possible role of MSDC in the tissue healing response also has not been described, but it cannot be excluded considering the various roles of mature myeloid cells in bone regeneration.[29,122]

The role of MDSCs in chronic implant infections seems analogous to those seen during the progression of tumors, wherein the accumulation of MDSCs leads to a tumor tolerant

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environment by suppression of proinflammatory responses in macrophages and T cells.[119] Several inhibitors of MSDC activity have been developed as an anticancer therapy. For example, all-trans retinoic acid shifts the differentiation of MDSCs into mature macrophages and dendritic cells, thereby removing the immunosuppressive effects.[123] Entinostat is a histone deacetylase inhibitor that suppresses MDSC activity and restores sensitivity of certain cancers to antitumor therapy.[124] Clinical trials are ongoing using INB03, an inhibitor of soluble TNF that inhibits MDSCs.[125] Whether the immobilization of implants with these agents converts them to anti-infective materials forms the basis of future research.

3. Anti-Infective Surface Modification Strategies

3.1. Bactericidal Nanopatterns

Recent advances in micro- and nanofabrication techniques have made it possible to endow implants with inherent bioactivity. Modifications confined to biomaterial physical properties are presumed to facilitate device regulatory approval and eliminate the possibility of resistance development compared to drug delivery systems incorporating conventional antibacterial drugs or growth factors.[126,127] As a potentially straightforward means to prevent bacterial implant colonization, specific patterned surfaces can be applied to directly kill bacteria via physical cues. Inspired by nature,[128] nanotopographical features such as nanotubes,[129] nanopillars,[130] nanospikes,[131] nanowires,[132] and nanoflowers[133] have yielded direct bactericidal properties. Although controversy exists about this topic, the underlying mechanism of killing likely involves stretching and rupture of the bacterial cell membrane upon contact,[134] whereby the sharpness of the nanopatterns[135] and the distance between the features[136] ultimately all determine the bacterial fate. Regarding the difference in mechanical properties of the bacterial cell membrane for different bacterial species, there is much uncertainty about the broadness of protection provided by bactericidal nanopatterns against common causative pathogens in IAI.[133] Moreover, as a current limitation, most of the bactericidal nanotopographies can only be applied to small-sized samples (i.e., 40–100 µm), which undermines progression to the (pre)clinical phase.[137] Consequently, the in vivo effectiveness of these surfaces is still unknown.

3.2. Influence of Surface Biophysical Cues on the Performance of Anti-Infective Biomaterials

High aspect ratio nanotopographies are usually less optimal for eukaryotic cell-instructive purposes since these surface topographies are several orders of magnitude below that of cells.[138,139] On the other hand, immune and osteogenic cells are highly sensitive to microscale topographies they come in contact with. Increasing evidence shows that surface topological patterns dictate their responses over other surface properties such as chemistry, hydrophilicity, and stiffness.[43,83,140,141] Substantial research has been conducted to better understand material-induced osteogenesis (reviewed by Gui et al.[139]), with

the final goal of improving the osseointegration of orthopedic implants. While the in vitro mechanoregulation of osteogenic cells has yielded strong effects, in vivo, osteogenic cells can only arrive at the biomaterial once postsurgical local inflammation has resolved or possible bacterial burden has been mitigated.[33] Therefore, it is critical to consider the possible host–biomaterial surface interactions that would predominate during the acute inflammatory phase. The next sections discuss how material biophysical cues affect macrophages and neutrophils, as they are the predominant cell types in contact with the biomaterial immediately after implantation.

Due to their plasticity and broad immunomodulatory functions, the selective polarization of macrophages using surface cues has been the subject of intense research.[104,142] Topological cues induce cytoskeletal network remodeling and formation of focal adhesions to affect the polarization and phagocytic role of macrophages, irrespective of chemistry.[104,138] Accordingly, the immunomodulatory effects mediated by different microtopographies—e.g., rods, fibers, pits, grooves, or pores—are mainly due to the respective changes in cell shape they induce. Several studies collectively show that a topography that endows macrophages to adopt an elongated morphology, such as parallel microgrooves, induce M2 polarization and anti-inflammatory cytokine production, while enhancing their phagocytic capacity.[143–147] Of note, these studies have either used zymosan particles or polystyrene beads to assess the phagocytosis rate,[144,146,147] which does not necessarily reflect their true potential to kill free-living bacteria.[109] In agreement, following in vivo implantation, microgrooves in the size range of 1–5 µm are associated with balanced inflammation and limited foreign body response compared to other microtopographies.[138] Using the TopoChip platform, comprising a library of over two thousand micropatterns to screen for proinflammatory, anti-inflammatory, or immune regulatory surface topographies, it was found that the pattern area of surface micropillars determines the extent of human macrophage attachment, while a combination of pattern area and density of the micropillars is crucial to instruct their inflammatory phenotype.[148] In the future, this platform may also help to answer how phenotypic changes in macrophages correlate to their anti-infective performance, as this is yet unknown.

In spite of their indispensable role in the anti-infective response, surprisingly little is known about the effect of surface topography on neutrophil behavior. Neutrophils are by far the dominant cell type to adhere to titanium implants exposed to human whole blood.[41] Although the detailed mechanisms are largely still unknown, recent evidence suggests that the biophysical influence of a biomaterial on neutrophil survival and function has been underappreciated to date. Chang et al. showed that potentially immune-compatible materials are turned into neutrophil-killing ones when microtopographies are introduced, whereby nonapoptotic cell death is preceded by markedly increased ROS production.[84] Similarly, other studies have demonstrated that microtopographies induce harmful NETosis in neutrophils in direct comparison to bioinert surfaces.[41,43] Moreover, there are data to support that the presence of a biomaterial might favor aberrant NET and ROS-mediated tissue damage over the clearance of infection by neutrophils, following a size-sensing mechanism.[101]

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The biomaterial-induced cell changes in neutrophils resemble those seen during frustrated phagocytosis in macrophages,[85] whereby the fate of neutrophils on a biomaterial follows a size-sensing mechanism: in response to small phagocytosable particles, the successful initiation of phagocytosis downregulates NETosis and maintains ROS production in the intracellular location, blunting further excess inflammation. However, when particles are too large to be phagocytosed, as is the case with permanent biomaterial surfaces, increased NETosis, extracellular ROS burst, and further neutrophil recruitment leads to an uncontrolled inflammatory response.[101,149] In particular the excessive NET formation and insufficient clearance of NETs is thought to underlie the aberrant inflammation in certain immune disorders or the foreign body response.[150,151]

As a relatively unexplored topic, a biomaterials stiffness is a second important biophysical cue that could affect the anti-infective functions of phagocytes. Increasing the stiffness of hydrogels enhances macrophage phagocytic ability and increases their proinflammatory cytokine production.[152–156] In neutrophils, material stiffness has opposite effects on their NETosis and phagocytosis.[83,140] Since load-bearing orthopedic implants have several multitude greater elastic moduli (in the GPa range[157]) than those usually tested in vitro (in the kPa range), it cannot be excluded that they would drastically impact the anti-infective responses from a biophysical point of view.

The effects of surface biophysical cues on neutrophil phagocytosis and other anti-infective properties warrant close investigation in the future, as they usually override the influence of material chemistry or protein adsorption.[43,83] **Figure 2** summarizes the many pathways by which topography can impact the host anti-infective and osteogenic responses, and illustrates that the biomaterial biophysical performance may have been an overlooked aspect in the design of anti-infective implants until now. For example, in clinical practice, orthopedic implants are often surface roughened to improve their mechanical anchorage to bone,[158] whereas the application of surface topography in the microrange is thought to promote osteogenic cell
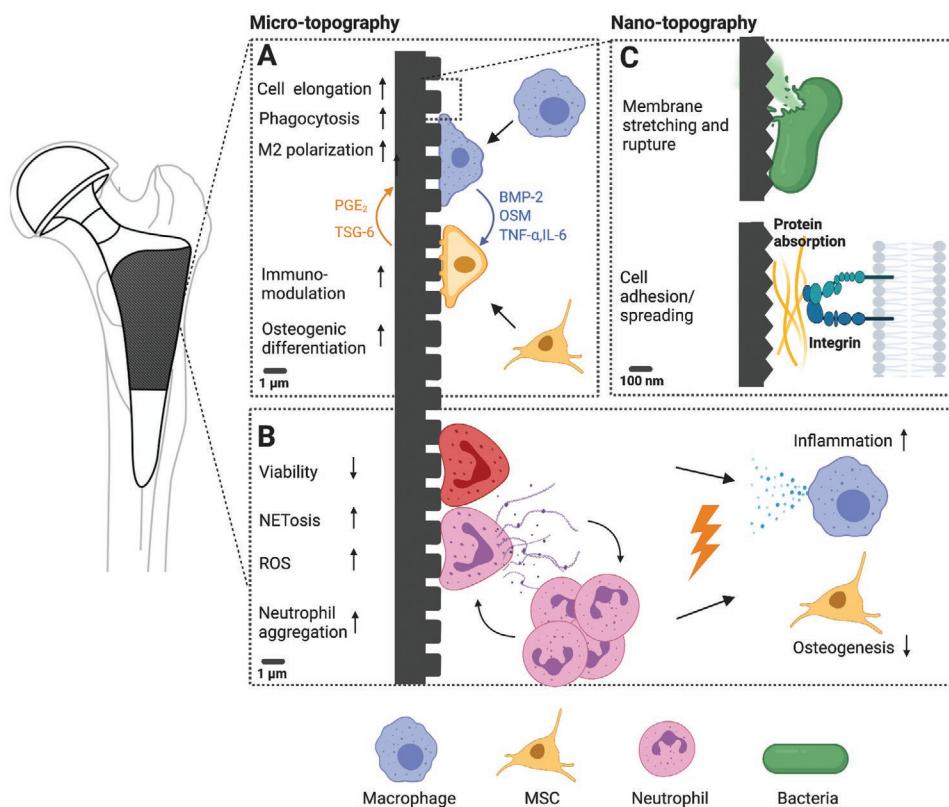

Figure 2. Influence of biomaterial surface topography on anti-infective and osteogenic responses within a host. A) Surface microtopographies that promote an elongated cell shape enhance the osteo-immunomodulatory crosstalk between MSCs and macrophages. In particular, parallel microgrooves induce M2 polarization and anti-inflammatory cytokine production in macrophages, while enhancing the phagocytic capacity. Nanopatterns in the same size range can modify either the osteogenic and/or immune regulatory behavior of MSCs. Accordingly, in the MSC-macrophage crosstalk, MSCs secrete paracrine factors such as PGE2 and TSG-6 to enhance the anti-inflammatory and pro-osteogenic phenotype in macrophages. B) Microroughness or rationally designed microtopography cause nonapoptotic cell death in neutrophils and promotes their exaggerated inflammatory state through mediating NETosis and extracellular ROS release. Moreover, chemotactic signals are produced that result in local accumulation of neutrophils. C) At the nanoscale, different topological features such as nanotubes, nanopillars, nanospikes, nanowires, and nanoflowers have shown direct bactericidal effects on both Gram-positive and Gram-negative bacteria, whereby the presumed underlying killing mechanism is the stretching and rupture of the bacterial cell membrane upon contact. Nanopatterns also modulate cell adhesion, spreading, and immune modulation in eukaryotic cells through changes in protein absorption and integrin-mediated mechanotransduction. The effects of nanopatterns on the anti-infective functions of host cells are still unknown.

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differentiation (Figure 2A).[141,158] It can be questioned whether such treatments influence the immune protective role of host immunity, and consequently contributes to their susceptibility to IAI. Moreover, the dysregulation of neutrophils around specific biomaterials could affect their crosstalk with other immune cell subsets and osteoprogenitor cells (Figure 2B).[33,42,88,104] It is already known that topography guides the communication between MSCs and macrophages, whereby enhanced secretion of immune regulatory factors including prostaglandin E2 (PGE2) and TNF-stimulated gene 6 protein (TSG-6) by MSCs promotes macrophages to establish an anti-inflammatory and pro-osteogenic milieu (Figure 2A).[33,159–163] A better understanding of the relevance of biomaterial contact guidance in anti-infective properties in host cells could lead to avenues that aim to promote host protection. Several pharmacological agents are known to revert the surface-induced deficiencies in neutrophils,[84,90] and could be harnessed for the sake of improved anti-infective performance. In addition, novel fabrication techniques increasingly allow the application of specifically designed nanotopographical surfaces. Although these methods are not suitable for the scale-up needed for actual therapeutic applications, they have drastically improved the biological understanding of stem cell and immune cell behavior in ECM-like 3D environments.[164,165] Nonetheless, the anti-infective behavior of nanopatterns, or their compatibility with bactericidal nanopatterning approaches, remains elusive (Figure 2C).

3.3. Influence of Material Chemistry in Immune-Instructive Biomaterials

In addition to the biomaterial surface topography, "stage-dependent" host immune responses can be strongly upregulated by biodegradable implants and their degradation products. As recently reviewed in detail,[166] the host responses to degradable materials are dependent on the class of material and the conditions opposed to the material at the specific implant location. In the case of commonly used synthetic polymers (e.g., poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA) and polycaprolactone (PCL)), small molecule degradation products activate the host response by engaging pathogen-recognition receptors or activating dendritic and T cells,[166,167] while local pH changes activate complement system.[168] In comparison, the byproducts of the popular natural polymer silk are far less immunogenic, and typically induces a mild macrophage-propagated inflammatory response that restores over a period of several weeks.[166]

Recently, the chemical properties of biomaterials have received much attention for the rational design of "immune-instructive" biomaterials, and it was shown that triazole surface modification of hydrogels hinders foreign body reactions in vivo and can potentially increase the longevity of implants.[169] The extensive role of surface chemistry in the quest for immunomodulatory biomaterials has been overviewed elsewhere (e.g., ref. [170]) and is therefore beyond the scope of the current review. For biodegradable metals such as Mg, Fe, or Zn, a combination of metal and hydroxide ions, and eroded particles stimulate the host response (see Section 4.3.1). Additionally, interfacial calcification precipitates further shape the host repose.[171] Although biodegradable metallic products often yield

favorable host response, it is crucial to consider the actual degradation kinetics at the implant site in the design and manufacturing of immunomodulatory biomaterials. For instance, Mg ions promote new bone formation by stimulating periosteum-derived stem cells;[172] nonetheless, a burst release of Mg can impede the required acute host response for tissue healing, for example by the low levels of reendothelization established.[173]

4. Immunotherapeutics and Their Biomaterials Application

4.1. Immunomodulatory Drug Release Systems

A second approach to produce anti-infective implants comprises drug release systems that timely orchestrate the local host response, and range from direct immobilization of drugs to the biomaterial, to systems for sustained and tunable drug release. Delivery systems following a direct antibacterial mode-of-action have been reviewed elsewhere.[15,21,174] Instead, the following sections discuss immunomodulatory surface engineering strategies, covering the advantages and limitations of different classes of immunomodulatory agents. More specifically, the utility of host defense peptides, metallic particles, and immunological adjuvants is reviewed in terms of their anti-infective effects and their potential for implant biofunctionalization. Finally, suggestions are made for combinatorial and bacteria-responsive approaches that can optimally address the current clinical need.

4.2. Host Defense Peptides

4.2.1. HDP Biological Functions

HDPs are naturally occurring peptides belonging to the host innate defense system and are predominantly upregulated during inflammation at epithelial surfaces and within neutrophilic granules.[175] As regulators of the inflammatory and anti-infective response, HDPs are regarded as interesting broad-spectrum alternatives to antibiotics in the prevention of IAI.[176] Due to their strong cationic charge, they cause a direct disruption of the microbial cytoplasmic membrane.[177,178] which can already be evident minutes after bacterial contact with HDPs.[179] As a second mode-of-action, selective HDPs can dysregulate intracellular targets in bacteria underlying their cell membrane integrity and replication.[180] These nonspecific killing mechanisms result in an extremely broad antimicrobial action against different bacterial/fungal strains and capsular viruses.[175] The different charge in mammalian and bacterial cell membranes is presumed to ensure a selectivity in toxicity toward bacteria over host cells.[181] Although conclusive evidence on this topic is lacking, it is argued that bacteria are incapable of developing resistance to HDPs, which is highly advantageous for their prophylactic use in biomedical devices.[176,182] Taking natural HDPs as the template, much effort is being devoted to producing derivative synthetic peptides with enhanced anti-infective properties, improved stability, or lower production cost, all features needed to facilitate their large-scale clinical implementation.[182,183]

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During the last decade, there has been a noticeable appreciation of HDPs as orchestrators of inflammatory and regenerative processes, alongside rising skepticism regarding their actual antibacterial effectiveness. Several observations have motivated this paradigm shift. First, the direct antibacterial effects of HDPs are severely dampened when exposed to physiologic conditions, such as the presence of host cells,[184] plasma components,[185] or physiologically relevant salt concentrations.[186] Moreover, several HDPs without any demonstrated antibacterial effects in vitro, can still clearly protect against infection in vivo.[159,187] Finally, the antibacterial effects of HDPs are usually seen at supraphysiologic concentration, while in contrast, several immunomodulatory, anti-biofilm, and wound-healing properties are apparent at concentrations resembling those found in body fluids.[188] In the following sections, we summarize the recent understandings in the indirect anti-infective effects of naturally occurring HDPs (i.e., LL-37 and human defensins) and synthetic innate defense regulators (IDRs) in light of biomaterial-based immunomodulation. The potential to harness them in biomaterial-controlled release systems is highlighted, as this is still a relatively unexplored field of research.

4.2.2. LL-37 and Derived Peptides

Cathelicidins are a family of small cationic peptides that are critical in the innate immune response to infections.[188,189] hCAP18 is the only known human cathelicidin, and is cleaved to obtain the bioactive peptide LL-37. LL-37 and its synthetic derivatives have been intensively studied as anti-infective HPDs, and are undergoing clinical testing phase as broad range antimicrobial agents.[187] As a major limitation of LL-37, only a narrow therapeutic window exists in which it exerts antibacterial effects without killing host cells. For example, cytotoxicity issues of LL-37 have been reported for concentrations as low as 10×10^{-6} M, whereas LL-37 in the range of 10×10^{-6}–200×10^{-6} M is needed for robust antibacterial efficacy.[190–194]

In spite of numerous anti-infective effects of LL-37 or their derived peptides reported in vivo,[188,189,192] their antibacterial effects in vitro are most apparent only at supraphysiologic concentrations (>30 μg mL^{-1}),[195] i.e., concentrations exceeding those found in innate immune cells and mucosal epithelial linings.[188,189] Consequently, it can be questioned whether anti-infective properties of LL-37 are indeed a consequence of direct bacterial killing. On the other hand, several immunomodulatory features of LL-37 are observed at concentrations far below those that kill or inhibit growth,[195] which could all improve the anti-infective response around a biomaterial.
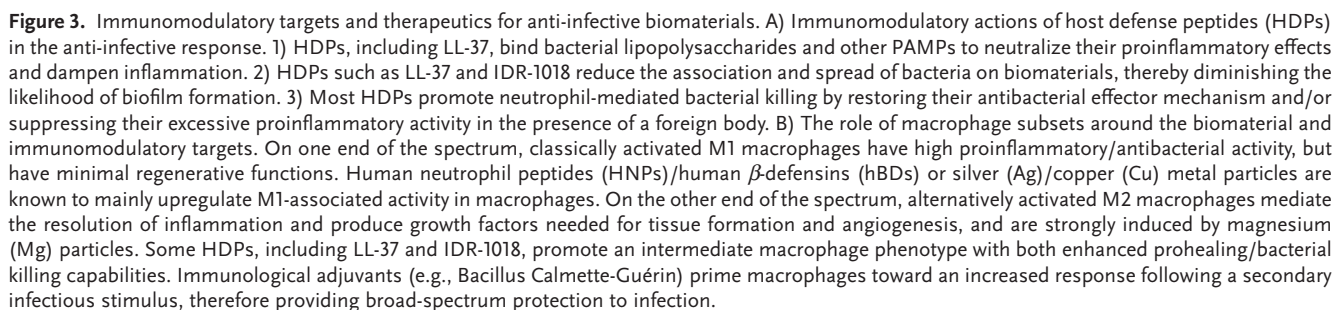
With respect to its immunomodulatory properties, LL-37 facilitates the onset of the anti-infective response through modification of chemokine and proinflammatory production[196,197] and promoting monocyte to macrophage differentiation.[198] This early inflammatory milieu propagated by LL-37 is furthermore characterized by the enhanced antimicrobial activity of macrophages and neutrophils.[199,200] Particularly of importance for prevention of *Staphylococcal* infections, LL-37 regulates NETosis and ROS production in neutrophils,[201,202] while protecting against biofilm formation by reducing bacterial attachment and spread on the implant.[195,203]

Given the dysregulated inflammatory response around a foreign body material, LL-37 may serve another important role in regulating the balance between protective and destructive elements of inflammation, while preserving the anti-infective environment.[175] In the presence of bacterial infection, LL-37 or their derived peptides prevent against collateral damage caused by excessive inflammation,[175,204,205] either by directly neutralizing cell-wall-associated pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharides (LPS) and lipoteichoic acids,[192,206] or by downregulating the cytokine storm that would otherwise result from hyperinflammatory macrophages (**Figure 3**A).[175,207] In line with these findings, it was found that LL-37 can restore LPS-induced bone loss in vivo.[208] Moreover, LL-37 is thought to stabilize the defective functions of neutrophils that normally are observed around the biomaterial.[28,89] In view of the "frustrated" inflammatory state around the biomaterial,[27] there is evidence to support that LL-37 favors the differentiation of macrophages toward a wound healing and prorepair phenotype,[100,209] without impairing their ability to perform antimicrobial functions (Figure 3B).[199] The immunomodulation by LL-37 could also promote the required macrophage-osteoblast crosstalk around the biomaterial needed for osseointegration.[208,210,211] This is a combinatorial advantage of LL-37 that could further imply its use to improve the functionality of orthopedic implants.

4.2.3. Innate Defense Regulators

IDRs are a class of short, synthetic peptide variants related to the bovine HDP bactenecin, and may potentially serve as broad-spectrum prophylaxis for infections. Notwithstanding their lack of direct bacterial killing abilities, IDRs are being screened for their anti-infective and immune regulatory properties.[212] First, IDRs promote leukocyte recruitment, driven by local chemokine production and integrin-mediated cell adhesion.[213] This leads to both a strong local accumulation of neutrophils and macrophages, but also an enhancement of their bacterial killing properties in terms of HDP release, chemokine production, and augmented phagocytosis.[213,214] Second, IDRs are known to repress potentially harmful inflammatory reactions without interfering with the immune anti-infective response (Figure 3). In neutrophils, several IDR peptides (i.e., IDR-HH2, IDR-1002, and IDR-1018) can suppress LPS-mediated inflammation, which coincides with improved neutrophil-mediated killing of *Escherichia coli*.[204,213] The IDR-1 peptide selectively inhibits the NF-κB pathway in macrophages, without dampening MAPK activity required for chemokine and anti-inflammatory cytokine production. These immune-protective effects of IDR-1018 on innate immunity have led to better control of infection in vivo.[159,214,215]

Of the various IDRs, some unique characteristics of IDR-1018 make it a key candidate to acquire in anti-infective biomaterials. In comparison to LL-37, IDR-1018 causes relatively little cytotoxicity and may even improve cell viability.[193] Moreover, since biofilm-formation is a primary reason for the recalcitrant complications associated with IAI, an important argument in favor of IDR-1018 concerns its potent anti-biofilm properties to a wide range of pathogens.[212,216] These anti-biofilm properties

Figure 3. Immunomodulatory targets and therapeutics for anti-infective biomaterials. A) Immunomodulatory actions of host defense peptides (HDPs) in the anti-infective response. 1) HDPs, including LL-37, bind bacterial lipopolysaccharides and other PAMPs to neutralize their proinflammatory effects and dampen inflammation. 2) HDPs such as LL-37 and IDR-1018 reduce the association and spread of bacteria on biomaterials, thereby diminishing the likelihood of biofilm formation. 3) Most HDPs promote neutrophil-mediated bacterial killing by restoring their antibacterial effector mechanism and/or suppressing their excessive proinflammatory activity in the presence of a foreign body. B) The role of macrophage subsets around the biomaterial and immunomodulatory targets. On one end of the spectrum, classically activated M1 macrophages have high proinflammatory/antibacterial activity, but have minimal regenerative functions. Human neutrophil peptides (HNPs)/human β-defensins (hBDs) or silver (Ag)/copper (Cu) metal particles are known to mainly upregulate M1-associated activity in macrophages. On the other end of the spectrum, alternatively activated M2 macrophages mediate the resolution of inflammation and produce growth factors needed for tissue formation and angiogenesis, and are strongly induced by magnesium (Mg) particles. Some HDPs, including LL-37 and IDR-1018, promote an intermediate macrophage phenotype with both enhanced prohealing/bacterial killing capabilities. Immunological adjuvants (e.g., Bacillus Calmette-Guérin) prime macrophages toward an increased response following a secondary infectious stimulus, therefore providing broad-spectrum protection to infection.

are mediated irrespective of any direct antibacterial mechanism, but through poorly understood mechanisms, IDR-1018 directly interferes with the association of bacteria with the biomaterial surface (Figure 3A).[212,216] IDR-1018 may also help ensure the balanced inflammation needed around the biomaterial for an optimal host defense.[159] IDR-1018 steers the differentiation of monocytes toward macrophages with broad immunomodulatory functions, reflecting both the classically activated (M1, proinflammatory) and the alternative-activated (M2, anti-inflammatory, regenerative) phenotype (Figure 3B). Such an intermediate phenotype would be particularly beneficial to circumvent the fibrotic response and promote wound healing and

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osteogenesis.[33,114,193,217] Importantly, these immune regulatory functions do interfere with the normal proinflammatory activities required for the anti-infective response.[116] As a short 12 amino acid peptide, IDR-1018 seems to easily penetrate tissue to target intracellular receptors in host cells, as demonstrated by its efficacy in soft tissue infections.[159] Hence, it can be hypothesized that IDR-1018 is also competent in targeting infection residing in the peri-implant tissue.[218–220] In agreement, Choe et al. demonstrated the immunomodulatory effects of IDR-1018 in a murine *S. aureus* IAI infection model. Intraperitoneal IDR-1018 injections before and after surgery promoted macrophage recruitment to the site of infection and blunted the excess production of proinflammatory cytokines, altogether leading to reduced infection and better osseointegration.[186] In spite of these encouraging findings, it should be carefully determined whether the strong suppressive effects of IDR-1018 on neutrophil activity form a potential danger for implant integration.[193,212,213] As a key component of the anti-infective and bone regenerative response,[42,98] a complete dampening of neutrophils around the biomaterial could lead to serious deficits in the anti-infective response.

4.2.4. Defensins

The humans defensins represent two subfamilies of cationic HDPs: 1) *α*-defensins, also referred to as human neutrophil peptides (HNPs), and 2) human *β*-defensins (hBDs). The defensins are expressed at low levels in normal physiologic conditions but are upregulated in response to infection, rendering them with antimicrobial properties against Gram-positive and Gram-negative bacteria, fungi, and viruses.[221] As with most HDPs, the antibacterial effects of the defensins are blunted in the presence of physiological salt concentrations and serum proteins,[222,223] and only a thin line exists between the concentrations at which they induce antibacterial activity or cell toxicity.[224,225] Together, these factors compromise their direct antibacterial use clinically.

With respect to their immunomodulatory functions, there is accumulating evidence that, in contrast to LL-37 or IDR-associated peptides, human defensins cause adverse effects in the host response to biomaterials. In the absence of a foreign body, high HNP levels in the intracellular phagolysosome contribute to the bacterial killing by neutrophils.[93] Alternatively, when neutrophils become associated with a biomaterial and acquire an activated state, they may secrete vast amounts of extracellular HNPs, leading to eukaryotic cell toxicity and reduced neutrophil anti-infective efficiency.[89,93,226,227] This is thought to, in part, underlie the dysregulation of incoming neutrophils around the biomaterial and their compromised phagocytic killing of pathogens such as *S. aureus*.[89,93] Similarly, it is known that hBDs amplify pathogen-associated immune responses and promote proinflammatory cytokine production.[213,228,259] hBDs also prolong the survival of activated neutrophils, which could further cause the accumulation of cytotoxic and proinflammatory mediators around the biomaterial.[230]

The potential contribution of human defensins as anti-infective agents requires more investigation.[213,221] The existing evidence highlights predominantly undesirable proinflammatory processes initiated by human defensins that could drive the immunopathogenesis of frustrated phagocytosis around the biomaterial. Moreover, possible contributions of HNPs or hBDs in the wound healing response around the biomaterial are largely unaddressed, while pro-osteogenic effects have not yet been reported. These findings discourage the use of human defensins in implant surface biofunctionalization strategies to prevent IAI.

4.2.5. Strategies for HDP Biofunctionalization of Biomaterials

Based on the current literature overview, HDPs can be designated as promising immunomodulatory agents to biofunctionalize implants. **Table 1** summarizes the advantages and limitations of the use of HDPs in the development of immunomodulatory biomaterials. As a distinctive feature of HDPs, their anti-infective mechanisms can be separated from cytotoxic or proinflammatory activity, which facilitates cell-mediated clearance of pathogens with only the necessary amount of inflammation. The use of controlled release systems together with combinatorial peptide delivery offers opportunities to fully address the key immune players in the course of the anti-infective and tissue healing response. As depicted in Figure **5**A, HDP delivery preferably should first aim to maximize the anti-infective effectors of neutrophils (i.e., phagocytosis, ROS production, and NETosis) in the critical postsurgical hours to days, while attenuating the chance of neutrophil-mediated inflammation. As argued before, an initial burst release of LL-37 (derived peptides) may ideally achieve this. Subsequently, the delayed and sustained release of mediators is needed that downregulate the neutrophil response and steer the differentiation of monocytes toward macrophages with anti-infective and immunomodulatory functions needed for onset of wound healing and osteogenesis (Figure **5**A). Current literature indicates that a delayed release of IDRs such as IDR-1018 over a period of days to weeks could be best applied for this purpose.

The state-of-the-art in implant biofunctionalization strategies using HDPs has thus far predominantly focused on their direct antibacterial effects. To illustrate, various HDP immobilization techniques render the HDPs with direct antibacterial activity, albeit under the prerequisite that flexible spacers support a certain motility of the peptide needed for its interaction with the bacterial cell wall.[231–237] In a similar fashion, this direct conjugation method likely also supports the anti-biofilm properties of certain HDPs by "shielding" the implant from bacterial colonization. For example, Gabriel et al. conjugated carboxylic acid poly(ethylene glycol) (PEG) spacers onto Ti implants by silanization, which allowed for subsequent covalent attachment of LL-37 specifically via its N-terminus.[235]

Immobilization techniques could become ineffective in light of most other anti-infective mechanisms of HDPs, where targeting host cells is more important than targeting bacteria. Instead, the immunomodulatory functions of HDPs necessitate their controlled release into the surrounding tissue, followed by their binding to bacterial PAMPs or cell-surface and intracellular receptors in host cells.[206,238] Several methods have been described for the controlled release of HDPs from metallic implants. For example, He et al. used polydopamine

Table 1. Overview of available immune modulators with respect to their advantages and limitations for implant biofunctionalization in anti-infective strategies.

	Advantages	Limitations
Host defense peptides	Direct antibacterial effects at high concentration	Toxic at supraphysiologic concentration
		Direct antimicrobial effects are antagonized at physiologic conditions
	Immunomodulatory functions at physiologic concentrations	Limited stability in vivo
	Regulators of macrophage- and neutrophil-mediated killing	Limited tissue penetration
	Inflammation suppressive effects	Intracellular targeting hampered by cationic nature
	Prohealing effects	
	Anti-biofilm activity	High cost of production
	Synergistic interaction with antibacterial agents	Limited preclinical evidence for IAI prevention
	Pro-osteogenic effects described	
Metallic particles	Direct and broad-range antibacterial effects	Toxicity for eukaryotic cells
	Modulation of bacterial killing and pro-osteogenic functions of macrophages	Immunomodulatory mechanisms are linked to inflammation
	Straightforward techniques available for immobilization or incorporation in bulk material	Effects on neutrophil functions poorly studied
	High stability, low cost	
Quorum sensing inhibitors	Specific targeting of bacterial virulence pathways	Broadness of targeting is limited; bacteria employ unique quorum sensing systems
	Bioactivity retained with different (covalent) immobilization methods	Evidence in implant infection models is still lacking
	Generally low toxicity	
Immune adjuvants	Clinically available	No direct antibacterial effects
	Broad protection against bacterial, fungal, and viral infections	Rarely investigated in context of IAI
	Multifunctional anti-infective/pro-osteogenic effects	Optimal timing of delivery unknown
	High stability, low cost	

as in interlayer on Ti implants to facilitate the biphasic release of LL-37 in vitro, that is, a burst release on the first day followed by a gradual release for 5 days. In vivo, this successfully resulted into improved preosteoblast recruitment and new bone formation.[210] Although polydopamine interlayers can be easily formed in a substrate and peptide-independent manner, the technology is compromised by the inability to tailor the release of a given peptide.[239] Alternatively, calcium phosphate (CaP)-based coatings allow better control over the HDP release. Whereas a thin hydroxyapatite (HA) film on Ti implants can already provide the electrostatic interaction with cationic HDPs to delay their burst release,[240,241] HDPs must be incorporated within a CaP coating for more sustained release profiles.[242–244] This has for example yielded 7 day steady release of the HDPs Tet213 and HHC-36 from CaP-coated metal implants.[244,245] Incorporation of HDPs in polymer-based coatings can enhance the in vivo stability of HDPs,[246] considering their susceptibility to proteolytic degradation or binding to plasma components.[4,246,247] For sustained immunomodulatory activity, He et al. immobilized LL-37-loaded silk fibroin nanoparticles (SFNPs) onto Ti by silanization and glutaraldehyde crosslinking. The resulting 7 day release of LL-37 improved the osteogenic behavior of MSCs and macrophages.[210] Since the electrostatic interaction of cationic peptide with the SFNPs forms the basis of the gradual drug release, SFNPs serve as a potential delivery tool for most HDPs. To realize even longer lasting drug release, multilayer polyelectrolyte assemblies can be applied to release HDP over a period of weeks.[243,248,249] For instance, Riool et al. produced implants coated with a polymer–lipid encapsulation matrix (PLEX) containing the LL-37-derived anti-biofilm peptides SAAP-145 and SAAP-276. The self-assembly of multiple alternating layers of polymer and

phospholipid in the PLEX provided zero-order release kinetics of the SAAP peptides stretching over a period of one month, and was more potent than an antibiotic-based doxycycline-PLEX coating in reducing the bacterial numbers.[243]

4.3. Metal Particles

4.3.1. Metal Particles as Double Edge Sword in the Anti-Infective Response

Metal particles including silver (Ag), copper (Cu), and magnesium (Mg), have a long history as antibacterial agents. Metallic Ag was already applied as an antibacterial compound before the discovery of antibiotics, and before microorganisms were identified as the source of infection.[250] Currently, metal particles still receive much attention in the design of bioactive materials, which stems from the opportunity to manufacture highly stable, inexpensive, and broad-spectrum antibacterial implant interfaces.[251] Moreover, many straightforward and effective biofunctionalization techniques have become available to incorporate metal particles onto metallic implants in different forms.[19,252,253]

The antibacterial mechanisms employed by metal particles have been studied in detail, showing that the release of ions is linked to the induction of ROS and cell toxicity. As a second antibacterial mode, metal NPs destabilize the bacterial cell wall through electrostatic interactions or by affecting the bacterial metal-ion homeostasis.[254,255] Although these modes-of-action underlie their broad-spectrum action, they also come at the cost of toxicity in eukaryotic cells, impaired phagocyte function, and damaging tissue inflammation.[38,252,255] The

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(harmful) immunomodulatory properties of metallic particles have been described in most detail in the context of wear particle disease, which causes loosening of prosthetic implants. In metal-on-metal or metal-on-polyethylene implant bearings, the gradual formation of micron-size particles during the wear process leads to inflammation characterized by an influx of different immune cells, production of numerous proinflammatory substances, and dysregulation of osteoclast activity, altogether leading to the loss of surrounding bone.[256] Importantly, the presence of wear particles also influences the risk of infection.[37,38] Although the underlying mechanism of this phenomenon is largely unknown, in the case of Co–Cr implant materials, it is reported that the wear products inhibit the rapid release of reactive oxygen species required for bacterial killing by neutrophils.[38] As reviewed elsewhere,[257] potentially harmful effects of metallic particle-induced inflammation should therefore be overcome, for instance, by using on-demand systems that timely respond to excessive inflammation and modulate the local tissue toward an anti-inflammatory, tissue repair environment. Since metal particles function as a double-edged sword in the anti-infective response, it is not surprising that their current clinical use is limited to only specific clinical scenarios.[258,259]

More recent evidence shows that metal particles stimulate endogenous anti-infective responses even when applied at subinhibitory concentrations.[260,261] Moreover, several observations have extended their biological actions toward proregenerative effects.[262,263] Foremost, pro-osteogenic effects are mediated by directly favoring osteoblast differentiation or priming macrophages in their crosstalk with osteoblasts.[264–266] As these are all key processes underlying the fate of a biomaterial, the use of metal nanoparticles as immunomodulatory agents could revive their application in future biomaterials design. Table 1 summarizes the advantages and limitations of using metal particles in the development of immunomodulatory biomaterials. The following sections discuss in more detail the immunomodulatory roles of Cu, Mg, and Ag in the design of anti-infective biomaterials, with special emphasis on the priming of macrophage and neutrophil responses.

4.3.2. Immunomodulatory Strategies Using Metal Particles

Copper: As therapeutic agents with antibacterial properties, copper ions (Cu^{2+}) are being investigated as biological component in metallic implants.[264] As a main challenge, the release of Cu^{2+} must be precisely tuned, since it can otherwise also cause cytotoxic and inflammatory responses.[264,267] Disregarding the toxic effects of Cu^{2+} on bacterial and eukaryotic cells, it is an important micronutrient needed for normal innate and adaptive immune functions.[268,269] In vivo, copper-deficient animals have an increased susceptibility to infections in association with compromised macrophage number and activity.[269] In vitro, physiologic Cu^{2+} levels support ROS-mediated anti-infective activity of phagocytes.[264] An immunomodulatory effect of Cu^{2+}-treated Ti implants was established by Huang et al., who employed a microarc oxidation technique to produce porous ceramic coatings incorporating Cu^{2+}.[260] The Cu-containing surfaces polarized macrophages toward a proinflammatory

M1 phenotype that enhanced their killing response directed against *S. aureus*. Of interest, this immunomodulatory response was seen at a Cu^{2+} concentration of 0.4 ppm, 1000-fold below its MIC+. In line with this finding, polyetheretherketone (PEEK) implants biofunctionalized with Cu using a sulfonation and magnetron sputtering technique, induced proinflammatory macrophage polarization with improved killing of MRSA both in vitro and in vivo.[270] Cu^{2+} may also serve a dual role, as biomaterial-mediated delivery of Cu^{2+} leads to favorable pro-osteogenic responses.[260,264] Together, this shows advantageous immunomodulatory features of Cu for its use in anti-infective biomaterials. Following surface biofunctionalization, the release of Cu should ideally be prioritized in the early response as it mainly induces a proinflammatory M1 shift in macrophages (Figures 3B and 5A).

Magnesium: Mg plays a crucial role in various physiological processes that could be harnessed in the development of multifunctional implants. As one of the essential inorganic components of bone matrix, Mg is crucial for the maintenance of normal bone health.[271] Early examples imply that Mg-based biomaterials are nontoxic and may actually stimulate bone tissue healing.[157,272,273] Together with its favorable biomechanical and in vivo degradation properties, Mg-based implants are finding their way into various clinical orthopedic applications.[157,274]

More recently, it has been shown that Mg exerts anti-inflammatory effects on macrophages, facilitating orthopedic implant integration. For example, the feasibility was for example shown by Li et al., who performed Mg coating using plasma immersion ion implantation. In vivo, Mg-biofunctionalized Ti implant induced more M2 macrophages, less inflammation, and decreased fibrous encapsulation as compared to bare Ti implants.[275] Similarly, MAO deposition of a Mg-containing ceramic layer onto Ti implants promoted a transition of macrophages from the M1 to the M2 phenotype, coinciding with decreased proinflammatory phenotype and increased osteogenic and angiogenic phenotype.[265]

Nevertheless, the immunomodulatory effects of Mg on key host defense functions requires closer examination. Although Mg does not affect the phagocytosis by monocytes/macrophages,[261] there are reports showing reduced phagocytosis[276] or oxidative burst[277] in neutrophils caused by Mg. Moreover, the anti-infective effects of Mg^{2+} have been questioned. Although the delivery of pure Mg protects against MRSA implant infection, Mg^{2+} has poor inherent antibacterial properties in vitro.[278] To not hamper the endogenous host defense response, it is suggested that the immunomodulation by Mg-based biomaterials should be delayed until after the acute postoperative period. At this point, Mg could help establish the anti-inflammatory environment needed to downregulate the foreign body reaction in favor of tissue regeneration (Figures 3B and 5A).

Silver: Ag is applied as an antibacterial agent in topical formulations.[252,258,279] However its use in permanent anti-infective biomaterials has been hampered by the marked toxicity of Ag ions (Ag^+) for mammalian cells,[252] for this reason there is ongoing research into improved Ag formulations and biofunctionalization techniques to reduce its toxicity.[280,281] Recent evidences highlight that immunosuppressive effects of Ag are

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an equally great concern.[282] Irrespective of Ag cytotoxicity, Ag impairs the phagocytotic response,[252] triggers the excessive release of NETs,[281,283] and induces atypical cell death in neutrophils.[284] Whereas Ag does not impact their phagocytic activity,[282] Ag exacerbates the inflammatory response in macrophages by inducing their M1 polarization,[285,286] ROS production, and NF-kB signaling-mediated secretion of proinflammatory cytokines.[287–289] The high reactivity of released Ag+ likely accounts for most of the immunomodulation by different Ag formulations, including various AgNPs.[282]

It can be argued that the harmful responses in phagocytes can counteract the normal host defense against pathogens, even when Ag is applied at nontoxic concentrations. Preclinical and clinical reports show that, paradoxically, Ag-doped biomaterials can in fact increase IAI rates through the sustained low-grade tissue inflammation.[252,290] Moreover, the long-term engraftment of biomaterials can be endangered by Ag-associated tissue destruction following high bacterial challenge. For example, AgNPs embedded in coatings were found to cause inflammatory osteoclast formation and exaggerated bone remodeling in an *S. aureus* bone infection model.[252,291] In line with this observation, it was found that Ag-coated implants induce significant release of elastase from neutrophils, impairing normal wound healing.[292] Together, this suggests that the broad-range immunomodulatory effects of Ag have been underappreciated in the biomaterials field. Specifically, the recent findings that Ag-doped biomaterials can render the neutrophils to become refractory against infection requires closer investigation.[252,292]

4.4. Targeting Quorum Sensing as an Anti-Biofilm Approach

The progression of bacterial infection from a planktonic to an adherent chronic state follows a coordinated expansion of the bacterial population, together with the upregulation of advantageous genes associated with virulence, immune evasion and biofilm formation.[293] During the various stages of biofilm formation, bacteria use QS as a local communication system to establish the necessary cooperative phenotype. At the molecular level, the secretion of bacterial autoinducers allows bacteria to sense and respond to changes in bacterial density accordingly.[293,294] Although following the same principle, there are differences in the QS systems utilized by Gram-positive and Gram-negative bacteria. Small N-acylated homoserine lactones (AHL) are the main autoinducer molecules in Gram-negative species, and are produced by a LuxI type protein, which binds to the LuxR protein. Gram-positive bacteria utilize larger autoinducer peptides (AIP). These AIPs are synthesized in the form of prepeptides, secreted via a specialized transport system, and are sensed by proteins with kinase activity, leading to phosphorylation of a response regulator protein.[295,296] Once a threshold of AHLs or AIPs are reached, the transcription of QS-regulated genes will provide the bacterial population with enhanced protection against the host's defense mechanisms.[293]

QS is closely associated with resistance to antibiotic and host-mediated killing;[297] hence the dysregulation of the normal QS systems is an important target in the development of biomaterials that can better control infection. The formation of biofilm goes through the stages of adhesion, microcolony formation, maturation, and dispersion. Several therapeutic QS inhibitors have been identified that can antagonize bacterial adhesion and biofilm formation, or disrupt biofilms by agonizing their enhanced dispersal into the free-floating form (**Figure 4**A).[295,298] Figure 4B summarizes the main QS therapeutic targets currently identified in anti-biofilm strategies: 1) inhibition of AIP synthesis (e.g., using RNAIII-inhibiting peptides),[299,300] 2) AI-2 pathway inhibitors (e.g., using fimbrolides and derivatives),[301,302] 3) blocking of autoinducer receptor binding or the signal transduction cascade (e.g,. using TrAIP-II),[294,303] and 4) inactivation of autoinducers (e.g., using enzymes degrading AHL molecules).[295,296]
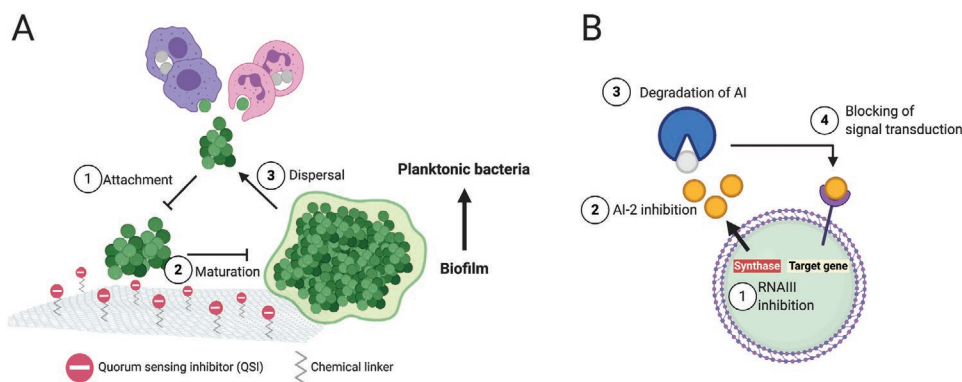

Figure 4. Inhibition of quorum sensing (QS) as anti-infective strategy. A) QS is closely associated with expression of the biofilm phenotype. The shift of bacteria from a biofilm to the planktonic state improves their sensitivity to antibacterial agents or host immunity. Using QS antagonists, inhibition of QS communication can reduce: 1) early attachment of bacteria on the surface or 2) the development of virulence needed for maturation of the biofilm. 3) Using QS agonists, the dispersal of implant-associated bacteria can be promoted. B) QS employs autoinducer molecules as a communication system, i.e., *N*-acylated homoserine lactones (AHL) in Gram-negative species and autoinducer peptides in Gram-positive species. The local concentration of autoinducers is proportional to the cell-population density. Once a threshold of AHLs or AIPs is reached, the transcription of QS-regulated genes will provide enhanced micro-organism with enhanced protection against the host's defense mechanisms. The main QS therapeutic targets currently identified in anti-biofilm strategies can be classified as: 1) inhibitors of autoinducer peptide (AIP) synthesis, 2) autoinducer (AI)-2 pathway inhibitors, 3) direct inactivation of AI by enzymes, or 4) blocking of AI receptor binding and the signal transduction cascade.

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Orthopedic implants most often fail due to *Staphylococcal* infections[3] with the accessory gene regulator (*agr*) QS system playing a key regulatory role in their pathogenic phenotype.[300] As a result, several anti-*Agr* compounds have been developed that could potentially be harnessed to render biomaterials with anti-infective properties.[298] For example, by repressing QS communication or triggering biofilm dispersal, the surface tethering of the *Agr* antagonist TrAIP-II or the AI agonist AIP-I, respectively, were successful in making biomaterials resistant to bacterial colonization.[294] The immobilization of the QS-targeting agents employed a click chemistry approach using azide-conjugated PEG surface linkers, proving that covalent attachment does not affect their bioactivity. Dihydropyr-rolones (DHPs) are synthetic, biocompatible derivatives of the fimbrolide class of QS inhibitors. DHPs inhibit the AI-2 family of autoinducers, and as opposed to most other classes of QS inhibitors, the AI-2 QS system is considered to be an interspecies QS system used by both Gram-negative and Gram-positive bacteria.[304] As a means to confer biomaterials with broad protection against biofilm formation, several studies have demonstrated that DHP reduce bacterial colonization when they are covalently immobilized. Ho et al. showed that covalent attachment of DHP to glass beads using a copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition reaction effectively reduces the adhesion of *Pseudomonas aeruginosa* and *S. aureus* up to 97%.[302] This biofilm protective effect was also confirmed in a subcutaneous in vivo infection model.[305] In a similar fashion, Ozcelik et al. used a PEG-based approach to produce DHP-coated surfaces with combined antifouling and anti-biofilm properties.[306] Small molecule inhibitors of RNAIII, i.e., the intracellular effector molecule that controls *agr* targets, have also shown to reduce the pathogenicity of *S. aureus* and *S. epidermidis*, albeit the role of surface functionalization of these compounds was not investigated.[299,300,307]

Whereas there is accumulating in vivo evidence demonstrating the benefit of functionalizing biomaterials with QS inhibitors, several challenges remain. Foremost, QS inhibitors have not yet reached large-scale clinical testing, and the possible development of bacterial resistance toward QS inhibitors is not clearly elucidated.[295] Also, there is still an ongoing search for QS inhibitors that offer broad targeting for infection prevention.[298] With the focus on *Staphylococci*, most of the current *agr* inhibitors block only one or two *agr* types, and inhibitors that can block all four *S. aureus agr* types still need to be developed.[307] In addition, *Streptococcus* spp. and *Enterococcus* spp. are also found in IAI, and their pathogenesis is regulated by largely distinct QS systems than *Staphylococcus* spp.[298,308,309] Finally, strategies that can destroy or remove autoinducers in the vicinity of biomaterials would be highly attractive.[295] Although several enzymes with such capacity have been identified, they only target AHL molecules secreted by Gram-negative bacteria and are therefore still of limited use in context of IAI.[295,296]

4.5. Adjuvants for Innate Immunity Training

The host recognizes microorganisms as "nonself" through a set of PRRs, of which the TLRs, nod-like-receptors (NODs), and C-type lectin receptors (CLRs) have been characterized in most detail. Whereas bacteria-derived lipids, polysaccharides or nucleic acids are mostly recognized by the former two classes of PRRs, fungal components such as *β*-glucans are recognized by the latter group of PRRs. The integrated response of the innate immune system to a pathogen depends on the combination of PRRs that are engaged, and tailors the production of cytokines, chemokines, and HDPs, needed for the first-line of defense against that microorganism.[310,311] Ligands for PRRs further modulate the antigen-specific responses of T- and B-lymphocytes through their interaction with antigen-presenting cells.[312]

Adjuvants are synthetic immunomodulators designed to harness the ability of PRRs to therapeutically enhance or suppress immunity.[313,314] A plethora of adjuvants, mostly targeting TLRs, are being clinically tested for their ability to enhance vaccine-specific responses, activate host immunity to exert anti-tumor or anti-infective effects, or suppress unwanted immune responses as in autoimmunity.[315] Immunological adjuvants can be considered a powerful class of immunomodulators to be incorporated into novel anti-infective biomaterials as an avenue for future research and practical applications. Due to the non-specific mode-of-action of immunological adjuvants, they can establish broad range protection to different microorganisms by employing cell recruitment, elevated phagocytosis, and enhanced ROS production.[316–319] Of the many available adjuvants, monophosphoryl lipid A (MPLA),[316,320] CpG oligodeoxy-nucleotides (CpG ODN),[320–322] and *β*-glucans[323] have proven to be particularly effective in protecting against *Staphylococcal* infections.[316,320,321,323]

Table 1 summarizes the advantages and limitations associated with the use of adjuvants in anti-infective biomaterials. Of the many uncertainties, it remains foremost to be determined whether the surface biofunctionalization of implants with adjuvants is feasible in the context of IAI. More specifically, comparative studies are needed to identify the adjuvants that lead to highest protection against causative pathogens in IAI, while causing the least adverse tissue reaction around the implant. PRR priming can either enhance or suppress the host anti-infective response to subsequent microbial challenge depending on the type of ligand, processes respectively referred to as "immune training" or "immune tolerance" (Figure 3B).[324] It can be reasoned that mild immunostimulants such as fungal *β*-glucans or Bacillus Calmette-Guérin can alleviate IAI as they prime the anti-infective response, whereas strong immunostimulants such as LPS could increase the susceptibility by dampening host immunity.[324–326] From a different perspective, there is evidence to support that the heightened inflammatory response resulting from immune training could exacerbate biomaterial-related inflammation and fibrosis, whereas LPS could act prophylactically to inflammatory fibrosis.[325] In the same line of reasoning, PRR ligands can differently impact the osteogenic response,[327–331] and it should be elucidated whether pro-osteogenic adjuvants also render implants with anti-infective properties. As a potential caveat, the innate immune training concept takes its advantage form the metabolic programming of host cells. As this requires several days to come into effect,[322,332] the elevated immune protection could be hampered in the first postoperative days, that is, when a surgery-related infection is most likely to occur. Of note, the priming of innate immunity may not be limited to PRR ligands, as it

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was recently shown that gold nanoparticles reprogram human monocyte to respond differently to a secondary microbial challenge.[333,334] Although this research avenue is still in its infancy, this suggests that sequestered particles or specific topological surface features on biomaterials are also contenders to enhance the antimicrobial response through innate immune training.

4.6. Resistance Development

The attractiveness of the aforementioned immunomodulatory drugs has followed their broad-spectrum effects, combined with a lack of negative association to antibiotic-mediated bacterial resistance. However, the inaccuracy lies in the notion that resistance occurs widely among various antimicrobials beyond only antibiotics. For instance, for Ag particles, resistance has been reported to stem from the production of neutralizing proteins that promote Ag particle aggregation or the formation of silver efflux network systems.[335,336] For HDPs, bacterial resistance can develop by means of efflux pumps or proteases, or the remodeling of the cell membrane.[182,337]

Notwithstanding the importance of further understanding these mechanisms, the fear of resistance becomes largely unfounded when the immunomodulatory agent exerts it anti-infective response by acting on host immunity, rather than on the bacteria. Moreover, in the case of HDPs, immunomodulatory effects are seen at physiologic concentrations, which is unlikely to lead to a selective pressure causing pathogen resistance.[187] Finally, immunomodulatory drug incorporation into anti-infective biomaterials is a prophylactic measure, as opposed to a biofilm treatment measure, avoiding long-term and repeated exposure to the immunomodulatory stimulus.

5. Converging Technologies in Anti-Infective Biomaterial Design

5.1. Combinatorial Drug Delivery

Since their discovery, antibiotics have been the gold standard in the prevention and treatment of infection, and no alternative therapeutics will likely overtake the role of conventional antibiotics in the near future. Realistically, multifaceted strategies can at best complement the available treatment modalities to more efficiently combat IAI and alleviate the reliance on antibiotics. The notion that the in vivo effectiveness of antimicrobials relies in part on the contribution of the host defense system, encourages the use of multipurpose biomaterials that target both the host and the bacteria.[338–340]

As a possible strategy to further explore, combined delivery of HDPs could improve the efficiency of antibiotics under certain scenarios. For example, through yet largely unknown mechanisms, macrophage-targeting IDR-1 has a synergistic effect together with the antibiotics cefepime or vancomycin in different in vivo infection models, even when the immunomodulator and the antibiotics are both used at their subeffective doses.[214] Likewise, the anti-biofilm peptides LL-13 and LL-17 have a synergistic action together with vancomycin. Remarkably, co-delivery of a subinhibitory concentration of these peptides

leads to a 100-fold reduction in required vancomycin concentration needed to kill *S. aureus*.[341] In terms of biofilm formation, the combinatorial treatment of IDR-1018 was found to synergistically decrease the minimal biofilm inhibitory concentration of antibiotics by 2- to 64-fold in a broad spectrum manner.[342] Apart from HDPs, certain therapeutic adjuvants such as β-glucans or phagocyte-targeting drugs have proven ability to enhance the effectiveness of antibiotics in vivo.[343–345] As depicted schematically in **Figure 5**B, it can be hypothesized that the combinatorial delivery of immunomodulatory and antibacterial may lead to optimal performance of anti-infective biomaterials.

5.2. Bacteria-Responsive Approaches

Although the absolute prevalence of IAI undoubtedly is high, IAI fortunately only occurs in the minority of patients receiving an implantable device. Since the current discussion focuses on biomaterials functionalization strategies as prophylaxis, an adaptable strategy that considers the different demands, namely, in cases with or without bacterial infection, would be ideal. To further complicate the matter, bacteria originating from remote infected tissue or the bloodstream can cause delayed onset IAI even weeks to months after biomaterial implantation,[346,347] an event that would not be covered in the case of early drug release. This necessitates a delayed and responsive drug delivery system.

Several delivery systems have already been explored to delay the release of antimicrobials until the time of bacterial challenge, either triggered by bacteria-associated pH changes or enzymatic activity.[174,348–350] Such delivery systems have mostly made use of the swelling/deswelling behavior of polyelectrolyte coatings as molecular gates for drug release. This is exemplified by Pavlukhina et al.,[351] who employed layer-by-layer deposition of poly(acrylic acid) carrying gentamicin. At pH 7.5, the coating did not elute measurable amounts of gentamicin for 45 days, while gentamicin was quickly released in an acidic environment. Similarly promising results were obtained using poly(methacrylic acid)- and tannic-acid-based polyelectrolyte coatings.[352,353] Advances in nanomedicine have also facilitated the design of on-demand antimicrobial drug release systems.[354–356] For example, Wang et al. created mesoporous silica coatings biofunctionalized with β-cyclodextrin. In this system, the antibiotics stored in the coating could be unlocked in a pH or enzyme-sensitive manner. As a huge advantage relative to polyelectrolyte-based coatings, these "nanovalves" circumvent the possibility of premature drug release.[356]

With the above in mind, ongoing research into on-demand systems could provide a means to ensure the appropriate tissue response in answer to the local requirement around the biomaterial. As presented in Figure 5C, under noninfected conditions, the host response should be directed toward a moderate anti-inflammatory response to dampen potentially harmful inflammatory reactions (e.g., using IDR-1018 or Mg). Under infectious conditions, the reaction should lead to the upregulation of NF-κB and MAP kinase-related proinflammatory cytokines and rapid activation of host immunity, namely, using drugs that induce M1-macrophage polarization and activate neutrophils (e.g., LL-37 or Cu).[214]
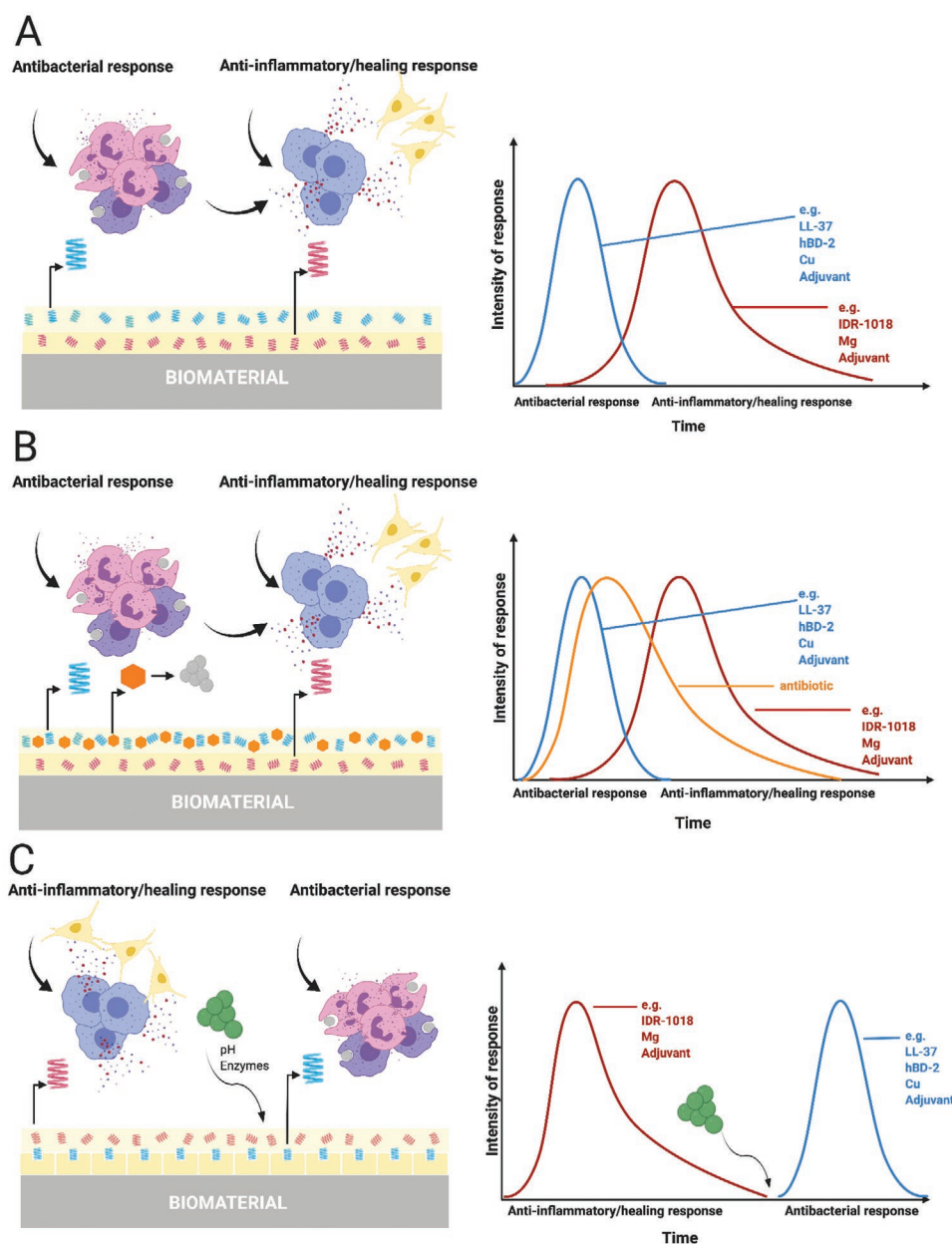
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Figure 5. Coating designs and representative drug release profiles. A) Passive approaches can deliver the immunomodulatory drugs according to predetermined release kinetics. For example, the sequential release of multiple immunomodulators needed in different healing stages can be tailored with multilayer coatings. With this approach, a burst release of an initial immunomodulatory agent can be prioritized in the critical postsurgical timeframe to propagate proinflammatory bactericidal activity, i.e., when the implant is at highest risk of bacterial colonization. This is followed by the propagation of an anti-inflammatory/wound healing response by the delayed release of a secondary immunomodulatory agent. B) The co-delivery of immunomodulatory and antibacterial agents can maximize the initial antibacterial response by targeting both the host and the bacteria. For example, immunomodulatory drugs and antibiotics often show a synergistic interaction in the anti-infective response. C) Bacteria-responsive strategies aim to circumvent premature drug release, but instead maximize the antibacterial response only when challenged by bacteria. The most common bacteria-responsive systems developed to date "unlock" in a pH or enzyme-sensitive manner in the presence of bacteria. Top: Coating composition and desired biological responses. Bottom: The corresponding drug release profiles envisioned for different coating compositions.

Recently, many other intelligent designs for implant biointerfaces have been explored for immune regulation, with most progress made in the field of cancer immunotherapy. Such designs may also have merit in anti-infection approaches, as they improve the therapeutic window of the immunomodulatory drug and are responsive to local inflammatory cues.

As an example of a fully controllable system, Chu et al. designed "immunodevices" composed of an ultraviolet light-activatable immunostimulatory agent (CpG ODN) together with upconversion nanoparticles. This system allows very precise spatiotemporal control of the immunomodulatory activity with near-infrared light.[357] Wang et al. developed an

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inflammation-triggered immunotherapeutic delivery carrier assembled by a long-chain of single-stranded DNA, designated as DNA "nanococoons." With these carriers, sustained release and synergistic action of anti-PD-1 antibody and CpG ODN was activated at the tumor site only under proinflammatory conditions.[358] Such a smart biomaterial can possibly be used to regulate the optimal inflammatory environment around anti-infective biomaterials. Finally, Tang et al. proposed the use of nanogels that deliver immunomodulatory drugs in response to changes in redox activity,[359] which could be suitable as a responsive system to redox-active bacterial metabolites.[360,361]

6. Concluding Remarks and Future Perspectives

The general presumption is that biomaterial implantation allows opportunistic bacteria to flourish by providing a surface for biofilm formation. With the "race to the surface" model in mind, anti-infective biomaterials have largely been linked to biomaterials with bacteria-repelling or bacteria-killing properties. Of equal importance in the pathogeneses of IAI, the work summarized here shows that most biomaterials promote a "frustrated" state in neutrophils and macrophages, characterized by excessive proinflammatory, and suppressed anti-infective capacity. The dysregulated host response contributes, in part, to the susceptibility of biomaterials to infection. Effective anti-infective biomaterials are therefore thought to provide optimal immune-protection with a minimal amount of necessary inflammation.

The off-target immunomodulatory effects of biomaterials will become an increasingly relevant topic when highly bioactive, and multipurpose materials are being favored over bioinert ones. This is exemplified by reports showing that antimicrobial agents with broad-range action, including silver or antimicrobial peptides, carry the danger of counteracting the normal host defense system, allowing infection to progress nevertheless. In fact, not only for silver, but for most immunomodulatory drugs, a narrow therapeutic window is thought to exist between their favorable anti-infective properties and the toxicity for host cells, including innate immune cells. Either their appropriate attachment to biomaterials or their controlled local release offer possibilities to reduce toxicity. It remains to be determined if their application at (near) physiologic concentrations can indeed provide immune protection while avoiding the cell toxicity and harmful inflammation often seen when applying them as an antibacterial agent.

It is stressed that neutrophils have been an underappreciated cell subset in the biomaterials field. Several lines of evidence show that they are rapidly dysregulated in the presence of a biomaterial, with possibly disastrous effects on cell survival, propagation of inflammation, and their ability to clear bacteria. Numerous studies collectively show that this process is likely governed by the biophysical mismatch between native body tissue and metallic implants. Considering that neutrophils stand at the frontline in the clearance of *Staphylococci* and other IAI-associated pathogens, we urge for closer investigation in the performance of these critical phagocytes in the development of novel anti-infective strategies, and stress the importance of the crossdisciplinary efforts needed for this.

HDPs could hold an important place within anti-infective biomaterials by holding potentially harmful inflammatory responses in check, while promoting bacterial clearance. Following this premise, LL-37- and IDR-derived peptides were identified as the most promising agents to instruct the necessary macrophage polarization and neutrophil bactericidal activity. This review also highlighted that metal particles and immunological adjuvants to have broad-range anti-infective effects. Although their utility as immunomodulatory agents still needs to be established with in vivo bone infections models, their established bone-promoting role favors their use for orthopedic implant biofunctionalization. As in vivo bone infection studies evaluating new immunomodulatory biomaterials often do not include control groups with antibiotics,[186,270,278,322] they cannot answer whether immunomodulatory approaches can achieve a similar efficacy as the current clinical standard. To further address the gap in the current literature, carefully designed in vivo studies should also aim at discriminating between direct and host-mediated pathways of bacterial killing by the anti-infective approach.

The overuse of antibiotics has resulted in the emergence of bacteria showing antibiotics resistance. This review enforces a proposed shift to multifaceted strategies that optimally select a high activity of host cells over bacteria to combat IAI and alleviate the reliance on antibiotics. Contrasting their traditional role as direct antibacterial agents, there is a need for surface biofunctionalization methods that optimally confer the immunomodulatory actions of HDPs, metal particles, or immunological adjuvants. Moreover, to address the clinical need and to mimic endogenous anti-infective responses, combinatorial and bacteria-responsive approaches are expected to perform better than monofunctional or passive biofunctionalization approaches. Several suggestions were made using polyelectrolyte hydrogel systems to ensure the appropriate tissue response in answer to the local requirement around the biomaterial.

As an important consideration for future research, the existing methods for in vitro testing of novel biomaterials require critical evaluation. The direct antibacterial/anti-biofilm activity and mammalian cell toxicity of a given biomaterial are accepted as the norms to demonstrate feasibility and cytocompatibility, but overlook the potentially disastrous alterations in host anti-infective ability. Hence, the use of overly simplified assays leads to an overappreciation of anti-infective effectiveness, and an unethical use of in vivo studies. As this is being realized, some efforts have been made to introduce the needed immunocompetence to in vitro assays. Li et al. validated a human-monocyte-based system to better recapitulate the course of the natural inflammatory response around a biomaterial, from initiation to resolution.[289] Using the model, AgNPs and AuNPs were found to exacerbate the response after challenge with infection-related stimuli (i.e., LPS, TNF-α, and IFN-γ) in the absence of toxic or direct proinflammatory effects. Likewise, Alsaleh et al. used macrophage and neutrophil-based assays to demonstrate impairments in essential host defense functions due to AgNPs, again in the absence of cytotoxicity.[362] Dalhoff et al. showed that the incorporation of macrophages or neutrophils in their model improved the predictive value when studying the pharmacokinetics of antibiotics.[363] In the pursuit of methods that can reliably identify immunomodulatory

biomaterials, it is urged that neutrophils will take on a more prominent role, as accumulating evidence shows that biomaterials downgrade or exacerbate key neutrophil effector mechanisms.[28,31,93,282] The current scarcity in neutrophil–biomaterial culture systems is most likely explained by the practical issues encountered while working with these cells and their short life span, ranging from 5 to 90 h.[364,365] As the necessary means to study critical neutrophil parameters—i.e., NETosis, oxidative burst, phagocytosis, and HDP production[28,31,93,282]—are not available in standard laboratories, this stresses the importance of crossdisciplinary efforts.

For unbiased screening and validation of immune-instructive biomaterials, high throughput methods such as microfluidics devices are extremely valuable, as they can be standardized and are more cost-effective.[366,367] As a matter of fact, in recent years, several high content analysis approaches have been developed to screen therapeutics for the induction of ROS and NET production,[368,369] and could be readily applied for testing of the neutrophil compatibility with biomaterials. Another example of an available high throughput approach is the TopoChip platform, which allows mathematically defined surface topographies to be screened for their influence on cellular responses.[141,148] As already validated for human macrophages, the combination of this platform with machine learning algorithms can lead to rapid identification of optimal immune-instructive biomaterial surfaces.[148]

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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