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Review Article

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Advances in dual functional antimicrobial and osteoinductive biomaterials for orthopaedic applications

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Abstract

A vast growing problem in orthopaedic medicine is the increase of clinical cases with antibiotic resistant pathogenic microbes, which is predicted to cause higher mortality than all cancers combined by 2050. Bone infectious diseases limit the healing ability of tissues and increase the risk of future injuries due to pathologic tissue remodelling. The traditional treatment for bone infections has several drawbacks and limitations, such as lengthy antibiotic treatment, extensive surgical interventions, and removal of orthopaedic implants and/or prosthesis, all of these resulting in long-term rehabilitation. This is a huge burden to the public health system resulting in increased healthcare costs. Current technologies e.g. co-delivery systems, where antibacterial and osteoinductive agents are delivered encounter challenges such as site-specific delivery, sustained and prolonged release, and biocompatibility. In this review, these aspects are highlighted to promote the invention of the next generation biomaterials to prevent and/or treat bone infections and promote tissue regeneration.

Key words: Antibacterial; Osteoinduction; Biomaterials; Orthopedic; Tissue engineering

Introduction

The design and development of novel biomaterials with dual function with both antimicrobial and simultaneous osteoinductive properties for various orthopaedic applications is highly desirable allowing for the treatment of infected and defected bone (Figure 1).[1,2] The treatment of infected/contaminated bone defects is a great clinical challenge promoting the prevalence of multi-antibiotic resistant organisms. In this context, microbial infections are a huge burden and a major challenge for society and public health leading to increasing healthcare costs. Generally, infected bone defects are treated with antibiotics prior to implantation, however, this approach is time consuming (can take several months to years)[3] and contributes to antibiotic resistance bacteria. On the other hand, implants associated with bacterial infections often require multiple operations, implant removal, long-term antibiotic use and rehabilitation. Therefore, having antibacterial properties integrated within the implant would reduce the time of healing and treatment, and total costs.[4] Furthermore, a localized infection in a bone injury or defect can result in limited bone healing.[5] To date, several materials with antibacterial properties have been developed such as silver nanoparticles (AgNPs),[6] cerium oxide nanoparticles (CeO_2NPs),[7] selenium nanoparticles (SeNPs),[8] copper (Cu),[9] polymers such as chitosan,[10] carbon nanostructures,[11] and antimicrobial peptides (AMPs).[12] They have further been employed in combination with the implant/biomaterial due to their good compatibility and broad

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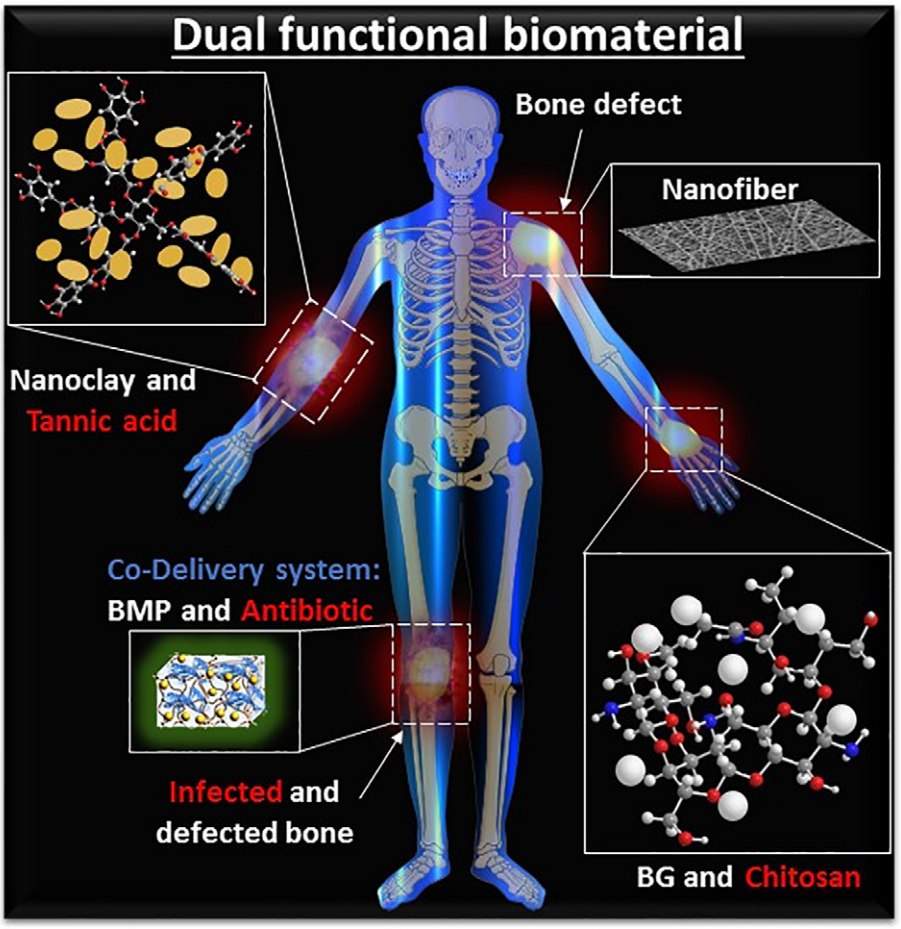

Figure 1. Examples of component with osteoinductive and antibacterial properties (dual functional) for various orthopedic applications that will be highlighted in this review.

bactericidal spectrum. Another class of natural based materials with a broad spectrum of antibacterial activity, but yet not fully explored, are polyphenol based polymers[13] (such as lignin[14] and tannin[15]) containing the important functional groups of gallol and catechol which contribute to their antibacterial properties.[16] Additionally, several materials and components have been employed for promoting bone healing and regeneration by inducing osteoconductivity and osteoinductivity, such as various elements (e.g. magnesium (Mg), zinc (Zn), strontium (Sr), silicon (Si), selenium (Se) and Cu),[17] bioactive glasses (BGs),[18] calcium phosphates (CaPs) (e.g. hydroxyapatite (HA), tricalcium β-phosphate (β-TCP), biphasic calcium phosphate (BCP)),[19] peptides,[20] and growth factors.[21] To address the *vide supra* stated limitations and challenges in an efficient, sustainable and cost-effective approach, novel biomaterials with dual action (that is, having the ability to promote bone regeneration and at the same time prevent any microbial infection) would be a great solution. In order to devise such an innovative material, the fundamental understanding of bacterial infections and osteogenesis is vital, which will pave the way for the development of a plethora of flawless materials. Here, advanced smart biomaterials with multifunctionalities[22] could serve as an important platform for bone tissue engineering applications.[23] Hitherto, many beautiful reviews on the antimicrobial and osteoinductive biomaterials have been demonstrated.[1–4,24,25] Nevertheless in this presented review we will try in a systematic approach, highlight the current challenges, advocate directions and strategies to solve various problems and limitations, which hopefully will benefit the readers in the engineering of next generation of biomaterials and advancements in antimicrobial and osteoinductive biomaterials for various orthopaedic applications. Initially, we start by briefly describing the fundamental basics of antimicrobial and osteoinductive pathways. Where vital aspects such as support of adhesion and localization of proteins, osseous cells, and growth factors from the implant, promote vascularisation, being resorbable (which allow the degradation of the implant in a controlled manner post implantation) will be highlighted. Additionally, having osteoinductive, osteogenic, osteoconductive, and the ability of bone-to-implant osseointegration are all essential for the engineering of successful and high-performance biomaterial. Furthermore, we give examples of various materials and components with osteoinductive and antimicrobial properties and then further highlight the few and recent reports on biomaterials with dual functionalities, emphasizing their applications with various biomedical challenges. Finally, we will discuss our future perspective and provide remarks to the reader concerning further

important information for advancing the field of dual functional biomaterials.

Current challenges

Bone tissue engineering has advanced continuously through the integration of biological, engineering, and clinical avenues.[26] Indeed, the first generation of prosthetics for use inside the human body was developed in the 1960s and 1970s, and underwent a critical shift that produced a second-generation of biomaterials by 1984.[27] This shift was accentuated by a decline in the clinical use of biologically inert materials and a correlative upsurge in research directed towards the fabrication of bioactive prostheses for tissue engineering applications. Bioactive materials implanted within a physiological environment are engineered to elicit essential actions and reactions with fine control. For instance, critical cellular functions including colonization, proliferation, and differentiation along implant surfaces are elevated by the selection of a biomaterial that is structurally and constitutively conducive towards these processes.

In the conceptual wake of bioactive materials having clinical benefits, second-generation biomaterials were adopted for applications in orthopaedics and dentistry.[28] These materials, which were primarily comprised of ceramics, glasses, and glass-ceramics, formed mechanically strong interfaces with bone tissue.[27] The incorporation of HA powders or coatings along metallic implants led to distinct osteoconduction.[29] By the 1990s, polymers were being adapted as bone prostheses, and the incorporation of bioactive compounds encouraged their application. For instance, HAPEX, which is a polyethylene and HA blend was recognized for its excellent applicability in middle ear bone repair and other replacement devices.[30] Besides satisfying the fundamental bioactivity condition, more sophisticated second-generation biomaterials were structurally and mechanically identical to bone tissue, in addition to being resorbable. The benefits of biomaterial resorbability were proficiently summarized by Hench in 1980.[31] A second method of manipulating the biomaterial-tissue interface is controlled chemical breakdown, that is, resorption of the material in the body.

Resorption of biomaterials appeared as a perfect solution to the interfacial problem because the foreign material is ultimately replaced by regenerating tissues. Ideally, there is eventually no discernible difference between the implant site and the host tissue. In a seminal publication in 1980, Hench characterized the influence of time on the resorption of Dexon sutures implanted subcutaneously within an *in vivo* rat model.[31] A significant 8-week reduction in the tensile strength of poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) composite sutures indicated good resorption and hydrolytic decomposition, and the clinical use of resorbable polymers as implant materials began to take effect.[31] Today, implant products with features such as bioinertness, bioactivity, and resorption have been approved for clinical applications by the United States Food and Drug Administration (FDA). Moreover, several companies that specialize in skin, bone, cartilage, kidney, cardiac, and retinal prostheses have found success through careful biomaterial designs.[26,27] Notwithstanding, research efforts have been directed towards improving biomaterials to overcome critical challenges that contribute to limited implant function or survivability. Due to the variability of contemporary scaffold devices, especially in terms of material composition, surface chemistry, pore size, porosity, morphology, degradation rates, and mechanical performance, controlled systematic studies are critical for eliminating complications that arise post-implantation.[26] A more sophisticated understanding of the microenvironment for osteogenesis would additively reinforce contemporary research aimed at designing the next-generation of bone engineering biomaterials.

Succinctly, researchers investigating osteoconductive and antimicrobial biomaterials are challenged with designing systems that are functional over a wide range of applications, biocompatibility, resistance to infection, unlikeness to promote microbial drug resistance, and antagonistic to a host immune response. A balance is often sought, in which efficacy (i.e., doing good) is not invalidated by safety concerns or harmful side effects (i.e., do not harm). Emulating nature does permit such an equilibrium.[32] Significant research efforts have produced complex scaffolds with inorganic, polymeric, or hybrid components.[26] However, modern technology has not resolved the dilemma of poor healing in bone tissue that is perforated by large critical-sized defects, and the effort to fabricate scaffolds with optimal mechanical properties and degradation kinetics has been complicated by the shear intricacy of physiological osseous matter.[33] Core challenges encountered in the design of bone tissue engineering scaffolds are highlighted in this section. The intricacies that govern the selection of scaffold material, design, influx species, and coating are evaluated, and technological factors for improving device adaptability post-implantation are considered. In general, the engineering of scaffolds for orthopaedic applications, in order to provide the best performance, they should display various properties presented in Figure 2, such as bioactivity, resorbability, antimicrobial, biocompatible osteoinductive, osteoconductive, osseointegration etc.

To fulfil all the requirements entailed a careful designed and choice of materials and strategies are needed. For instance, the selection of the base material is vital and normally encountered with the engineering challenges meeting the optimal mechanical and physical requirements matching the host tissue (Table 1).[29] To address the challenges bone derived components such as CaP, displaying similar mechanical properties as the native bone have been employed,[34] composite scaffolds composed of a combination of native derived material and polymers, thus taking the advantages of the distinct different materials to meet the required physical and mechanical properties of host tissue,[35,36] or another strategy could be by the addition of dopants such as silicon dioxide (SiO_2) and zinc oxide (ZnO).[37] Additionally important factors are the structural configurations and morphology such as porosity, pore size and surface topography of the scaffolds engineered which will promote the structural continuity and integrity of the scaffold and at the same time provide space for new tissue formation (Table 1). In this context, several materials with these properties have been designed and employed for various orthopaedic applications such as osteochondral graft,[38] PLGA film,[39] hollow electrospun PLA fibers coated with silicon-calcium-phosphate bioactive organic-inorganic glass.[40] Moreover, to improve the performance of the designed

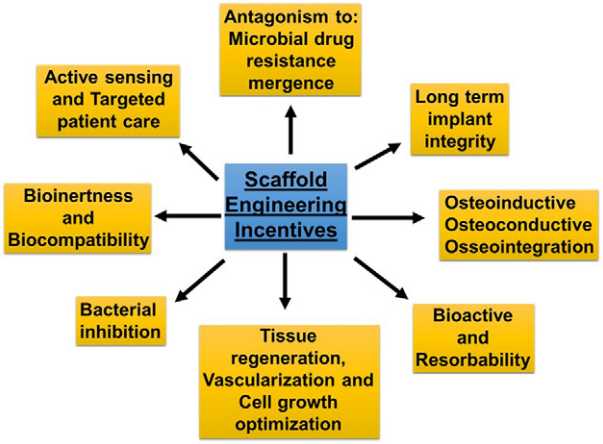

Figure 2. Demonstrating some of the motivations, visions and desired properties when designing and engineering biomaterial scaffolds with dual functional.

biomaterials further strategies could be the addition of agents such as cells, drugs, nanoparticles growth factors etc. or the performance of implant coatings. These strategies could allow the enhancement of the biological performance of the biomaterials/implants, promote tissue ingrowth and vascularization or the controlled delivery of the various agents and prevent/treat infections and defect. Within this context, various materials have been disclosed such as poly-*L*-Lactic acid (PLLA) device,[41] gelatine microparticles incorporated with agents,[42] BG ceramic 45S5,[43] bioactive and antibacterial CaP-based coatings,[44] Nitric oxide (NO)-based polyvinyl chloride (PVC) coated xerogel,[45] alginate hydrogel loaded with growth factors,[46] garlic extracts and oils as antibacterial agents[47] and titanium implants coated with silver titanium dioxide (TiO_2) and HA.[48]

The ability for an orthopaedic scaffold to support tissue attachment, regeneration, and proliferation is a central consideration in the design of bone growth implants. Since synthetic materials cannot respond to physiological stimuli to the same degree or in the same direction as living tissues, a conceptual shift has taken place in which medical practitioners now seek to regenerate rather than to replace native tissues.[27] This concept has been integrated into biomaterial designs. Nevertheless, one key element that has been recognized to counteract the functionality of artificial organs is insufficient vascularization.[50] Vascularization is a critical factor in the transport of oxygen and nutrients to cells localized in implant regions and in the transferal of damaging waste materials away from metabolizing cells. Although skin, ligaments, and cartilage have good accessibility to existing blood vessels,[51] large-scale three-dimensional (3D) tissues, such as bone, require a proximal blood supply to ensure survival, and they are as a result more difficult to engineer artificially.

To address these deficiencies, researchers have experimented with scaffold prevascularization and growth factor delivery.[41,42] Additionally, a direct correlation can be inferred between the porosity of a substrate and the localized blood influx; this correlation predicts a relationship between the scaffold porosity and vascularization.[52] Since bone scaffolds tend to have heterogeneous pore sizes, and therefore, variances in blood influx volumes, discrepancies in vascularization may be observed on a sample-by-sample basis.[52] Moreover, integration of the scaffold with the host vasculature is intrinsically very complex, and research efforts must be exerted to resolve this complication.[53] Figure 3 presents biomaterial modifications that have been explored to improve vascularization.[54]

Presently, implant surface features or intrinsic designs may heavily guide essential protein adsorption and cellular attachment mechanisms, in addition to supporting vascularization, which in turn would assist the proliferation of osteoblasts. Gao et al. and Karp et al. have previously reported the value of interconnected scaffold macroporosities for improving 3D cellular growth.[38,49] However, this finding is devalued by research indicating poor healing along such scaffolds that are devoid of living cells.[55] In other words, direct cell transplantation in implant models is often investigated to improve osteoconduction, although shortcomings do emerge with this auxiliary structural revision.[53] The most disadvantageous among these is the probability of poor adjacent vascularization, which would lead to inadequate intracellular nutrient and oxygen uptake, extensive cell death, and negligible cell-cell adhesion. Therefore, manufacturing surface features or elements that would elicit a predictable cellular response are additional primary design considerations.[27,56] Moreover, the nature or composition of a surface may promote the tailored derivation of specialized cell classes. As an example, consider the research performed by Bielby et al., in which murine embryonic stem cells demonstrated a penchant towards the osteogenic cell differentiation when grown along bioactive substrates that produced soluble ionic extracts saturated with Si and calcium ions (Ca^{2+}).[43] Indeed, scaffolds expressing improved *in vivo* biological effects due to the incorporation of living cells are constructed by virtue of rigorous analytical and experiential processes. Here, modern scaffolds are often enriched with design elements for supporting bone growth and eluding infection.

However, a major contributor to bone implant failure is bacterial adhesion and growth in the area of the implant. This can be caused by lapses in surgical hygiene, contact with the normal microbial flora, or the incursion of microorganisms, due to trauma, i.e., as in the case of compound fractures, to the site that is being operated on.[57] Over the long term, bacterial colonies residing

Table 1
Important factor impacting the performance of engineered biomaterials/implants and the various strategies to improve these features.

Influencing Factors	Engineering Challenges	Aspirations	Strategies	References
Base Material	Scaffold constitution optimal mechanical and physical properties	Match the host properties	1. Bone derived components e.g. CaP 2. Composite scaffolds 3. Dopant addition (e.g. SiO_2 and ZnO)	29,34–37
Structural Configuration and Morphology	Porosity Pore Size Topography (i.e. Nano roughness)	1. Structural continuity, integrity and stability at the scaffold/bone interface 2. Provide new space for tissue and blood vessels grow	1. Osteochondral graft composed of Hyaluronan derivative, MSCs and CaP ceramic. 2. PLGA film 3. PLA fibres coated with P_2O_5-CaO-SiO_2	38,40,49
Additive Agents and Implant Coatings	Cell Transplantation Pre-vascularization Drugs Nanoparticles Growth Factors Proteins Peptides Natural Medicinal Substances and Minerals Metabolic Rates Drug Release Profiles	1. Enhanced biological performance, promote tissue ingrowth and vascularization 2. Controlled delivery of agents. Prevent and treat infections and defects.	1. PLLA device 2. Gelatine microparticles incorporated with VEGF and BMP-2 3. BG ceramic 45S5 4. CaP-based thin coatings (F-CaP, Zn-CaP and F-Zn-CaP) with antibacterial and bioactive properties 5. NO releasing xerogel coated with PVC 6. Alginate hydrogel functionalized with RGD-peptide and incorporated with BMSCs, BMP-2 and TGF-β3 7. Garlic extracts and Ziziphora essential oil with antibacterial activity 8. Antibacterial Titanium alloy implants coated with silver, TiO_2 and HA	41–48

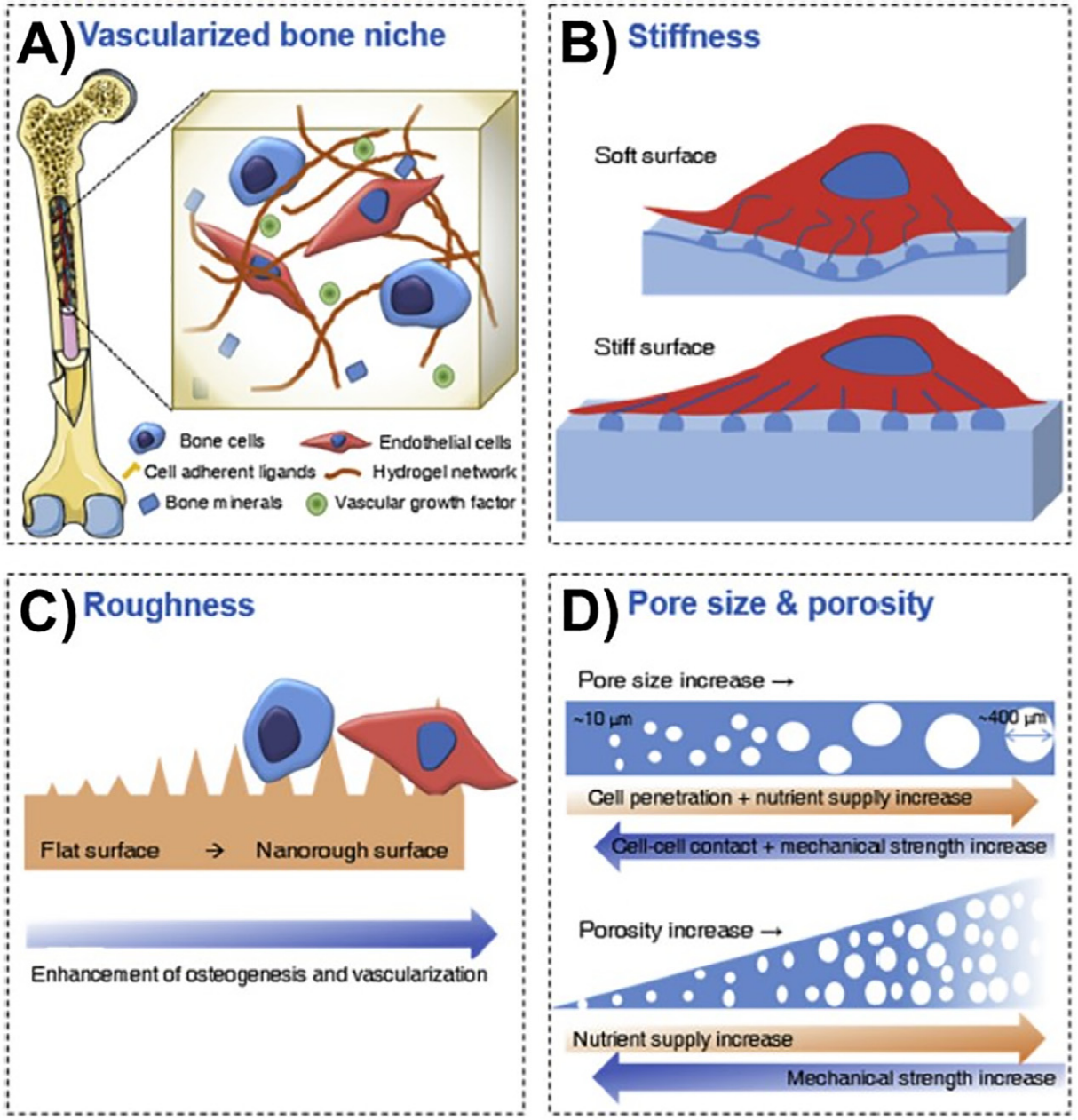

Figure 3. Various important biomaterials properties promoting osteogenic differentiation and vascularization, such as A) vascularized bone niche B) stiffness C) roughness D) pore size and porosity. Reproduced with permission.[54] Copyright 2019, Elsevier B.V.

along implant surfaces tend to propagate and form extensive biofilms, which would obstruct tissue integration and lead to significant patient trauma.[58] Infections often lead to severe patient suffering and pain, and their occurrence may necessitate urgent surgical intervention for implant replacement and localized infection removal. Moreover, patient compliance to 1-2 week antibiotic regiments would need to be pursued to improve the prospect of recovery.[59] However, the use of antibiotics may be disadvantageous. Antibiotic overuse has been credited with benefiting the livelihood of virulent bacterial strains that are drug-resistant, and a high probability exists that the cell source of infection is inherently unresponsive to antibiotics. For instance, methicillin-resistant *Staphylococcus aureus* (MRSA) is on the rise, and MRSA infection rates as high as 30% have been documented among accident survivors who have undergone bone fracture surgeries.[59] MRSA is exceptionally troubling due to protracted dormancies and the possibility of biofilm formation after up to 6 months.[59] Therefore, various solutions have been evaluated in an effort to diminish infection potential. Among these, the most prevalent are implant coatings and supplementation using antibacterial agents, proteins, drugs, or nanoparticles.[44,60] To account for possible dormancy complications and biofilm formation, a layer, coating, or additive must remain stable or intact over an extended timeframe. Commonly, silver components or nanoparticles are applied as coatings along implant surfaces due to their intrinsic antibacterial efficacies.[61] Conditions must be

Table 2
Selected bone graft substitutes and their resorption mechanisms. Reproduced with permission.[65] Copyright 2010, Elsevier Ltd.

Material Types	Materials	Resorption Mechanism
Ceramic	Bioglass	Very limited resorption by partial dissolution
	Calcium sulphate hemihydrate	Dissolution
	Gypsum	
	Dicalcium phosphate dehydrate	
	Calcium carbonate	Dissolution or cell-mediated
	Dicalcium phosphate	
	Octacalcium phosphate	
	β-Tricalcium phosphate	Cell-mediated
	Biphasic calcium phosphate	
	Precipitated hydroxyapatite crystals	
	β-Calcium pyrophosphate	
	Sintered hydroxyapatite	Practically no resorption
Metal	Magnesium	Corrosion
	Iron	Corrosion
	Tantalum	Practically no resorption
	Titanium	
Polymer	Polylactides	Hydrolysis
	Polyglycolides	
	Polycaprolactone	
	Cellulose	Transport to lymph nodes
	Hyaluronan	Hyaluronidase
	Fibrin	Plasmin
	Collagen	Collagenase
	Chitosan	Lysozyme

adjusted to regulate silver ion (Ag^+) release in controlled dosages over an intended timeframe within the region of the implant. Under ideal conditions, a silver coating must perform and remain active for a period of at least one year without imposing any significant native tissue damage.[59]

Similarly, conventional antibiotics, nitric oxide, and antibodies have been incorporated into BG, polymer, or HA biomaterials to reduce bacterial adhesion and prevent possible biofilm formation.[45] Other coatings (i.e., self-assembled monolayers) may be tailored to inherently block planktonic or biofilm cell attachment.[62] However, when meticulous consideration is overlooked, these designs may be linked to low success rates due to localized tissue toxicity and the potential for an associated upsurge in bacterial drug resistance. Such a discussion, which surveys the incorporation of drugs, nanoparticles, proteins, growth factors or diverse bioactive agents into scaffold device structures, can naturally be extended to the activation of osteoconduction and localized tissue growth.

Physical adsorption and covalent attachment modes have also been investigated for immobilizing bone morphogenic proteins (BMP) and transforming growth factor beta (TGF-β) along prosthetics and tissue engineering scaffolds to improve osseointegration and cellular functionalities.[46] Moreover, the addition of trace elements to orthopaedic implants has been associated with enhanced implant strength loss kinetics, mechanical strength, and biocompatibility properties.[59] Drugs and natural medicinal substances, such as Aloe Vera, turmeric, and garlic extracts, can additionally aid with bone regeneration, inflammation reduction, and Ca^{2+}-absorption when incorporated into orthopaedic biomaterials.[47,63]

Despite these advances, manufacturing a substrate that contains both antibacterial and osteoconductive constituents is by default incredibly challenging. Critical factors to evaluate when modelling a feasible biomaterial system that is coated or intrinsically loaded with antibacterial and/or osteoconductive agents include drug metabolic rates, *in vivo* dilution effects, and sustained post-implantation delivery in the device field.[26] It is additionally useful to develop clinical strategies that enable drug re-application to the biomaterial surface after implantation and before surgical wound closure.[26] Even more dismaying, many coating materials tend to exhibit limited flexibilities and covalent adhesion, and these properties are correlated with poor device presentation and restricted resorbability *in vivo*.[64]

Moreover, creating resorbable biomaterials is a task that in itself is inherently challenging. The shift towards introducing only resorbable materials and components into the human body is part of a movement towards achieving implant biocompatibility and reducing surgical disturbances. As resorbable elements are designed to degrade in a controlled manner after implantation, the absence of residual synthetic substances minimizes issues of autoimmunity or bacterial habitation, and the necessity for surgical intervention or implant removal is almost eliminated.[59] In the transition towards adopting resorbable substances as the principal biomaterials for bone healing applications, controlled strength-loss glasses and ceramics, including BG and CaP-based substrates, have complemented or replaced biopolymers with positive effects.[59] Indeed, a materials science and physiological challenge that stems from using resorbable materials is the regulation of degradation rates *in vivo* to match tissue ingrowth kinetics as the healing progresses. Factors that influence this dependency include the site of implantation, the nature and extent of the defect undergoing treatment, and fluid mechanic outcomes. Common bone grafting materials and their resorption mechanisms are summarized in Table 2.[65]

It is clear that a thorough understanding of cell-matrix interactions requires a research course that is sophisticated. Developments are underway to construct responsive and specialized scaffolds in addition to large-scale screening systems that can elucidate the impact of discrete structural modifications on scaffold functionalities.[26] Contemporary devices are evolving from passive instalments into active tools that monitor the healing process or remotely communicate malfunctions using wireless technologies.[59] Such devices are in the theoretical and primal phases of development, although very near to becoming clinical paradigms due to advances in sensor technologies. Ultimately, active prosthetics may eliminate the need for frequent X-ray or magnetic resonance imaging appointments, deliver drugs or active agents *in situ*, or electrically stimulate the formation of bone tissue by remote interference or direct impulse detection. Depending on patient needs, devices may be equipped with a range of specialized capabilities, and this transition towards individualized patient care has been branded personalized medicine.[66] Patient-matched devices are fabricated on an individual basis to aid in the treatment of critical defects that are complex or to improve patient healing rates and comfort. Here, technological advances have enabled 3D printing strategies such as direct metal laser sintering to be used in the fabrication of biomaterials that are uniform or unique.[67] One additional consideration in implantation research is the availability of systematic clinical tooling devices for avoiding irregularities in physician-to-physician surgical results. Robotic surgery is an attractive option for minimizing mechanical variations in procedures, and more advanced tooling procedures may be adapted for complex orthopaedic procedures.[59,68]

The fundamental basics of antimicrobial and osteoinductive properties

Bone disorders and injuries contribute immensely to significant societal strains, and medical intervention, including surgery, and are often urgent and inevitable. Orthopaedic deficiencies call for the restoration or replacement of skeletal tissue that is fragmented or otherwise degrading due to trauma or age-related tissue degeneration. Therefore, the primary intent of any orthopaedic implant is to support the adhesion and localization of proteins, osseous cells, and growth factors in the region of the bone defect. For any type of bone healing process, the induction of osteogenesis is guided by the recruitment of undifferentiated and pluripotent cells to the defect site, and this is referred to as osteoinduction.[69] During osteoinduction, immature cells acquire the preosteoblast phenotype before differentiating to form osteoblasts. Osteoconduction is a related phenomenon by which cell growth along a surface is evaluated. The basic definition of osteoconduction can be extended to include bone proliferation along biomaterial surfaces, in addition to bone conduction on the superficial layers of living tissues *in vivo*.[69] By current interpretations, therefore, osteoconduction occurs along and within the surfaces, channels, conduits, and pores of implantable biomaterials.

Implant materials of excellent biocompatibility are favoured for fabricating bone growth scaffolds, mainly due to their improved osteoconduction.[69] Once good osteoconduction has been established, osseointegration accounts for the assimilation of implant materials with physiological tissue, whereby the formation of bony tissue around the implant is unperturbed by fibrous capsule formation or poor osseous cellular adhesion. By default, specialized orthopaedic scaffolds must be osteoinductive, osteogenic, osteoconductive, and capable of bone-to-implant osseointegration to achieve success in the clinic. Although these concepts are interrelated, few distinctions may be advantageous or offsetting. For instance, an implant surface does not need to be intrinsically osteoinductive, since osteoinduction is a natural tissue response that will advance the bone healing process, regardless of the foreign material's ability to promote new bone formation.[69] In contrast, osteoconduction and osseointegration are strongly influenced by nature and the performance of the implantable biomaterial, and the focus of many researchers, therefore, is to stimulate bone anchorage to biomaterial surfaces, which would effectually enhance osteoconduction and supplement natural osteoinductive processes. Concepts related to osteogenesis, osteoconduction, and osteoinduction are represented in Figure 4, *A*.[70,71]

A fundamental secondary objective in developing scaffold technologies for bone defect applications is to deter bacterial attachment and growth along the biomaterial surface post-implantation. This is of particular importance since implant failures have been exceedingly linked to the formation of bacterial biofilms along implant surfaces *in vivo*, and in many cases, months after successful surgery. Although bacterial biofilm formation is often undetectable at the outset, the long-term effects may be catastrophic. Biofilm initiation occurs when isolated planktonic bacteria irreversibly settle on an implant surface and proliferate.[71] The proliferating organisms produce extracellular polymeric substances that promote phenotypic changes and the assemblage of a dense cellular matrix.[71] Lapses in surgical hygiene, likely propagated by the microorganisms commonly constituting the human microbiota such as *staphylococci*, together with compromised patient immunity, can incur severe microbial infections that would necessitate immediate medical intervention. To combat these interfering microorganisms, antibiotic or other drug treatments may be applied directly on the implant material prior to implantation, or be administered to patients as needed post-surgically. The chronic evolution of antibiotic-resistant bacterial strains, however, including MRSA, reinforces the need for transplantable devices that naturally impede microbial growth and biofilm formation, without the utility of specialized drugs or antibiotics.

In contemporary medical research, nanotechnology is envisioned as an absolute solution to countering the adverse impact that is often imposed by harmful microorganisms. For instance, nanofeatures and nanotopographies that are etched, annealed, or printed onto biomaterial surfaces have been proven to deter the attachment of bacteria.[48] Nanomaterial coatings have also been shown to restrict bacterial adhesion and growth.[72] Several processes account for outcomes that preclude the formation of biofilms on implants, whereby the nature of the active nanomaterial heavily guides surrounding antibacterial processes. With regards to nanotexturing, the surface roughness of a biomaterial plays a pivotal role in increasing the bone/implant

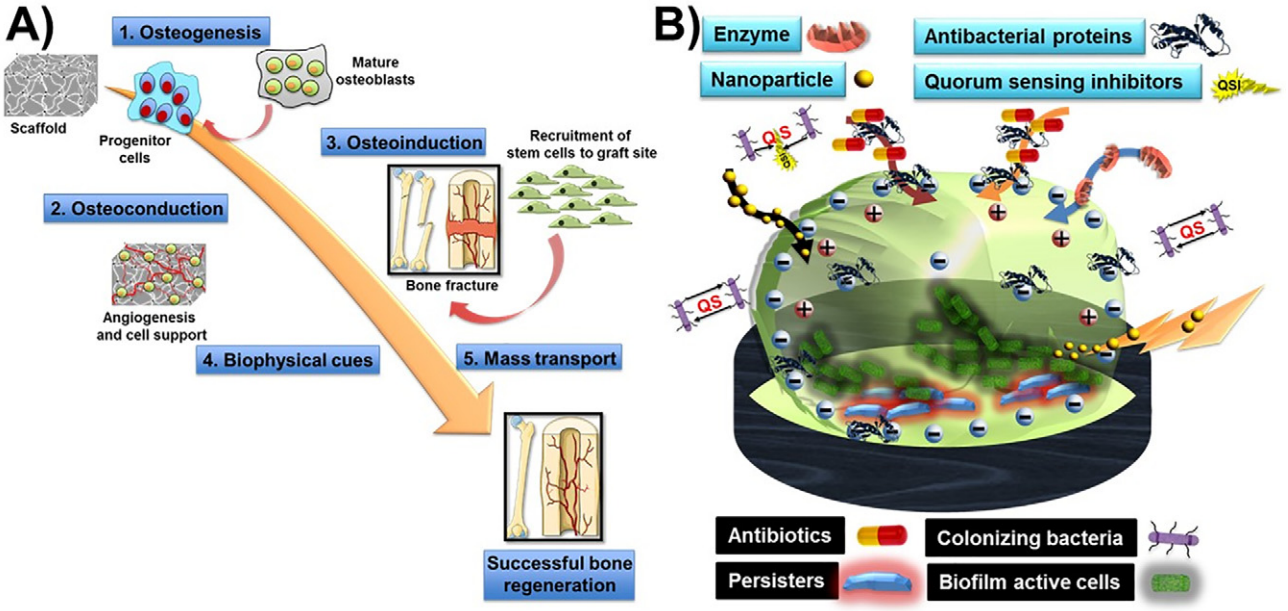

Figure 4. A) Osteogenic, osteoinductive, and osteoconductive processes in the vicinity of an arbitrary scaffold are depicted, and influencing conditions are referenced, with regards to *in vivo* bone regeneration and B) the alternative therapeutics for combatting bacterial biofilms are summarized.

contact area, maximizing bone on-growth, and upregulating osteogenic factors and protein secretion. Surface roughness further leverages the surface energy of a biomaterial. Optimized surface energies have been correlated with improvements in the local recruitment of osteoblasts and a reduction in the attachment and propagation of bacterial or fibroblast cells.[73] Furthermore, transition and lanthanide metal nanoparticles can be used as biomaterial coatings to directly suppress invading microorganisms by membrane disruption, reactive oxygen species (ROS) overproduction, or the perturbation of critical intercellular mechanisms (Figure 4, *B*).[74]

Antimicrobial biomaterials

Death from drug-resistant infections is estimated to cause a higher mortality rate than all cancers by 2050, leading to approximately 10 million deaths.[75] Within this context, the development of materials for biomedical applications with antimicrobial properties is essential to provide an alternative to traditional antibiotics. Nanomaterials have provided new weapons against bacteria accessing pathways not available for traditional therapeutics; hence they are promising candidates to overcome the current problems with antibiotic-resistant bacteria. Over the year, we have witnessed the frequent employment of AgNPs for various biomedical applications due to their broad spectrum of antibacterial activity.[76–80] The great potent antimicrobial activities of AgNPs is due to several mechanistic pathways, where it generally proceeds to kill bacteria through four different pathways: 1) the damage of bacterial membranes, 2) the inhibition of DNA replications, protein synthesis and enzymatic activity, 3) cellular toxicity and ROS generation and 4) through the alteration of cell respiration (Figure 5).[6,81,82]

Nevertheless, even though AgNPs have been employed successfully in various fields and for different biomedical applications, caution and awareness have to be considered since some studies have demonstrated bacteria developing resistance to silver.[83] Moreover, nanoparticles from other elements such as Se, cerium (Ce), gold (Au), titanium (Ti), Cu, iron (Fe), carbon (carbon nanotubes (CNT), fullerenes, graphene) have also been studied for their antimicrobial effects.[84] Here, Se in its nanoparticle form (SeNPs) has attracted great interest for a wide range of biomedical applications, especially as an antimicrobial agent.[8,85–88]

For instance, Huang and co-workers have disclosed the functionalization of SeNPs with both Quercetin (Qu), a bactericidal compound found in plants, and with acetylcholine (Ach), a neurotransmitter used as permeability-active unit in antibacterial compounds (Figure 6, *A*).[89] Qu–Ach@SeNPs particles exhibited a synergistically antibacterial performance against MDR superbugs, such as MRSA, at a low dose. Due to the presence of acetylcholine, the Qu–Ach@SeNPs gained the ability to attach to the bacterial cell wall, causing membrane disruption and leakage of the cytoplasm; posteriorly, the nanoparticles invade bacterial cells and disrupted the DNA (Figure 6, *B*).[89] Several other research groups have also successfully demonstrated the employment of SeNP as an antimicrobial agent.[90–92] Moreover, recently, it has also been demonstrated that CeO_2NPs display anticancer, antioxidant and bactericidal activity through the modulation of ROS levels and, may therefore be useful in biomedical applications to protect cells against radiation damage, oxidative stress, inflammation and contamination by microorganisms.[7,76,93] The mechanism for CeNPs bactericidal action was shown to be dependent on if the particles can be internalized by the cells. For non-internalized particles, but presenting direct contact with the cell membrane, toxicity was found to be associated with ROS generation, membrane disruption or interference with nutrient transport functions. Nevertheless, when ceria was internalized by the cells, the mechanism of death may involve lysosomal injury or oxidative

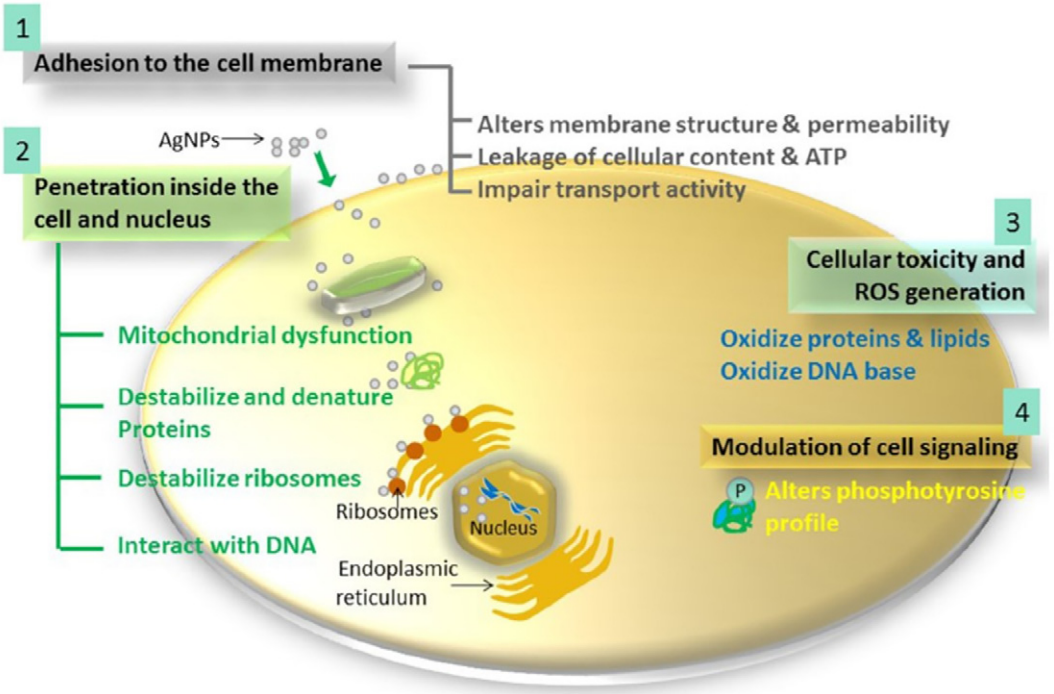

Figure 5. The general mechanism of action for the antibacterial activity of AgNPs proceeding through four approaches 1) Adhesion to the cell membrane and causing damage 2) penetration inside the cell and nucleus 3) generating ROS and causing cellular toxicity and 4) modulation of the cell signaling causing cell death. Reproduced with permission.[81] Copyright 2016, Dakal, Kumar, Majumdar and Yadav, licensed under A Creative Commons Attribution License (CC BY).

stress.[76] The authors investigated the antibacterial activity of 5 nm CeO_2NPs coated with dextran (a biocompatible polysaccharide), against *P. aeruginosa* and *S. epidermidis*, due to their significant prevalence in implant infections (Figure 7).[76] Dextran-coated CeO_2NPs were synthesized by fabricating a mixture of an aqueous solution of cerium salt and dextran with ammonium hydroxide, and the prepared particles were used to study their influence on the growth of bacteria.[76] Varying the dose, the time of treatment, and the pH of the solution, the authors concluded that dextran-coated CeO_2NPs were more effective at a basic solution (pH = 9) compared to having an acidic solution (pH = 6). After 6 h of treatment with nanoceria at pH 9, an intense reduction of bacteria growth and elevated amounts of ROS generation per colony were observed compared to the untreated controls, showing drastic morphological changes as a result of cellular stress.[76] Aside from the employment of nanoparticles, additional strategies, such as the use of natural polymers, synthetic polymers, and peptides have been demonstrated.[21,94] Antimicrobial biomaterials have been used to protect bacterial contamination of wound dressings, surgical sutures, bone cement, medical devices, implantable prostheses, among others; but in order to clinically apply these materials, it is essential to present low toxicity to mammalian cells.[95] Moreover, due to different carbon hybridization (e.g. sp^2, sp^3), many carbon nanostructures have now being explored and consequently have boosted developments in physics, chemistry, material engineering, biology and medicine since the 1990s.[96] Carbon structures such as Fullerenes,[97,98] CNT and Graphene (Figure 8).[11,96,99–101] The antimicrobial efficiency and mechanism of action depends on their intrinsic properties, such as composition, surface modification, size, nature of the target microorganisms, and characteristics of the environment in which cell-carbon nanostructure interactions take place.[11,102] Typically, the bactericidal action of carbon nanostructures involves a combination of physical and chemical mechanisms: (i) mechanical disruption, by structural damage to the cell wall and membrane of the microorganism, and (ii) oxidative stress, by the generation of toxic substances, such as ROS due to the high reductive potential of the nanostructures.[11,102–107] Nevertheless, pure carbon nanostructured materials are costly and, thus, unlikely to be broadly applied as optimal antimicrobial materials. Alternatively, they have been combined with polymeric, ceramic and composite materials while also providing antimicrobial activity. Aslan and co-authors have investigated the effect of single-walled NT (SWNT) with a diameter between 0.8 and 1.2 nm.[108] Previous report have also disclosed the combination of more than one carbon nanostructure providing bactericidal activity.[109] The reader is referred to some recent reviews discussing the bactericidal activity of carbon nanostructures for a deeper investigation and overview.[11,102,110] Additionally, antimicrobial polymers have either an inherent capacity to display antimicrobial activity, such as chitosan, compounds with quaternary nitrogen groups, *N*-halamines, and poly-ε-lysine, or they can be modified with biocides and antibiotics to present antimicrobial activity.[111] Polymers with quaternary ammonium groups are the most explored kind of polymer with inherent antimicrobial properties.[112] In this context, Yang and co-workers have summarized the design and synthesis of antimicrobial cationic polymers by controlling the cationic groups and the hydrophobic groups.[113] The antimicrobial activity can be tailored depending on the type, amount, location and distribution of these two components. Taking this into account, Gupta et al. have created polymeric nanoparticles that effectively

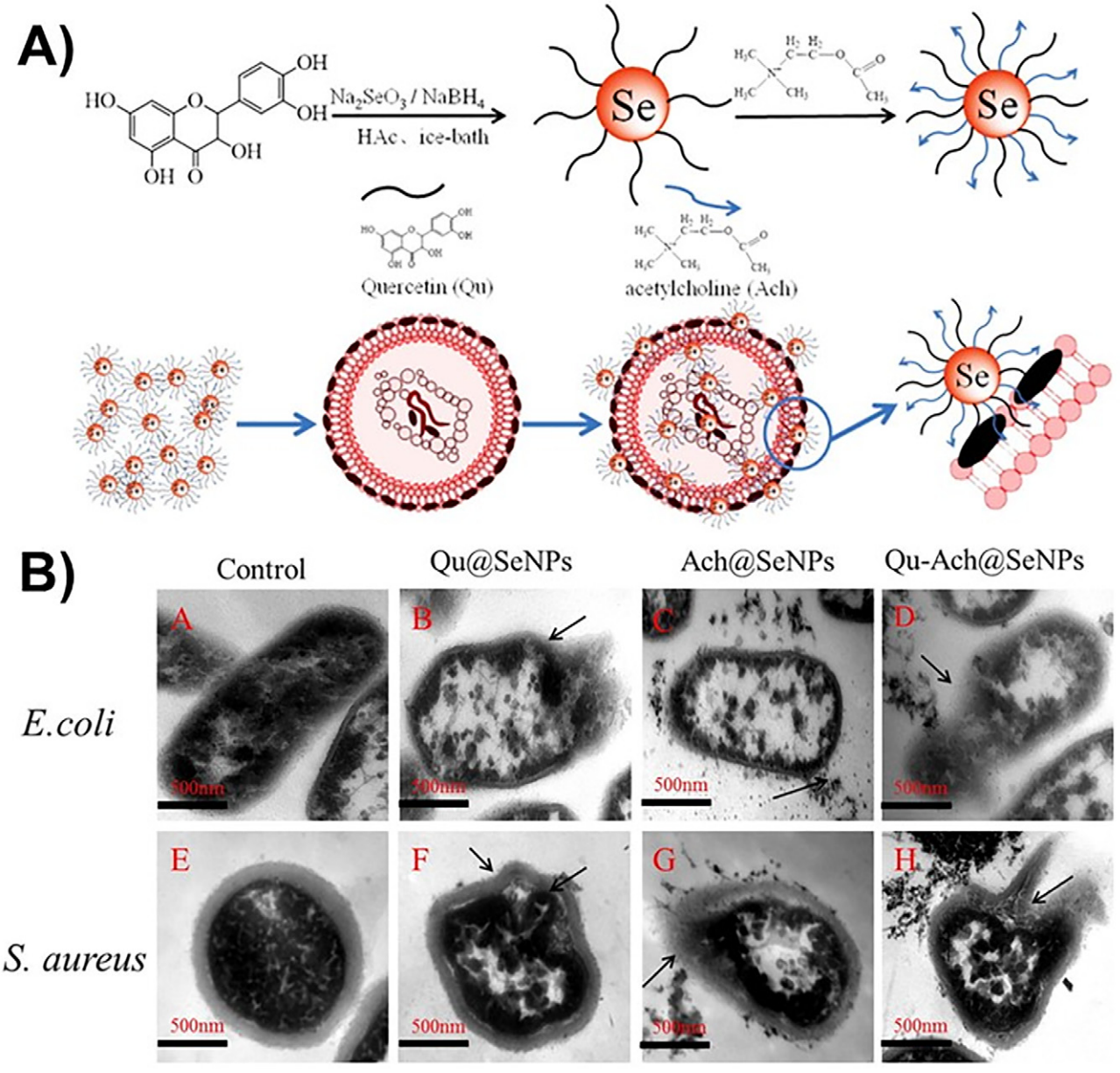

Figure 6. A) Synthetic scheme of SeNPs functionalized with quercetin (Qu) and acetylcholine (Ach) providing the Qu–Ach@SeNPs. B) TEM images of MDR *E. coli* and MDR *S. aureus* treated with Qu@SeNPs, Ach@SeNPs, Qu–Ach@SeNPs, and a control (without nanoparticles). Reproduced with permission.[89] Copyright 2015, Acta Materialia Inc. Published by Elsevier Ltd.

eradicate preformed biofilms.[95] This was performed through careful engineering of hydrophobic and cationic domains of quaternary ammonium poly(oxanorborneneimides), while maintaining low toxicity to mammalian cells and simultaneously avoiding bacterial resistance after 20 serial passages (Figure 9). It was found that oxanorbornene polymer derivatives containing a bridged C11 alkyl chain spontaneously self-assembled into cationic polymeric nanoparticles with a ~13 nm size as confirmed by transmission electron microscopy (TEM), dynamic light scattering (DLS) and Förster resonance energy transfer (FRET) experiments.[95] It was observed that longer hydrophobic alkyl chains greatly improve the bactericidal effect, eradicating preformed biofilms through a membrane disruption mechanism that strongly attenuates generation of tolerance or resistance.[95] Additional interesting work on antimicrobial cationic polymers have been reported by Du and co-workers.[114] In their study, injectable and biodegradable hydrogels utilizing poly(hexamethylene guanidine) (PHMG) and poly (ethylene glycol) (PEG) were fabricated through a thiolene "click" reaction. The PHMG-PEG hydrogel showed remarkably low toxicity and a broad-spectrum of strong antibacterial activity.[114] Enduring, chitin, poly(β-(1→4)-*N*-acetyl-*D*-glucosamine), is a natural polysaccharide present as a structural component in the exoskeleton of arthropods or in the cell walls of fungi and yeast.[115] At an industrial scale, chitin is mainly obtained from crab and shrimp shells, by acid treatment to dissolve calcium carbonate followed by an alkaline extraction to solubilize proteins, and then a

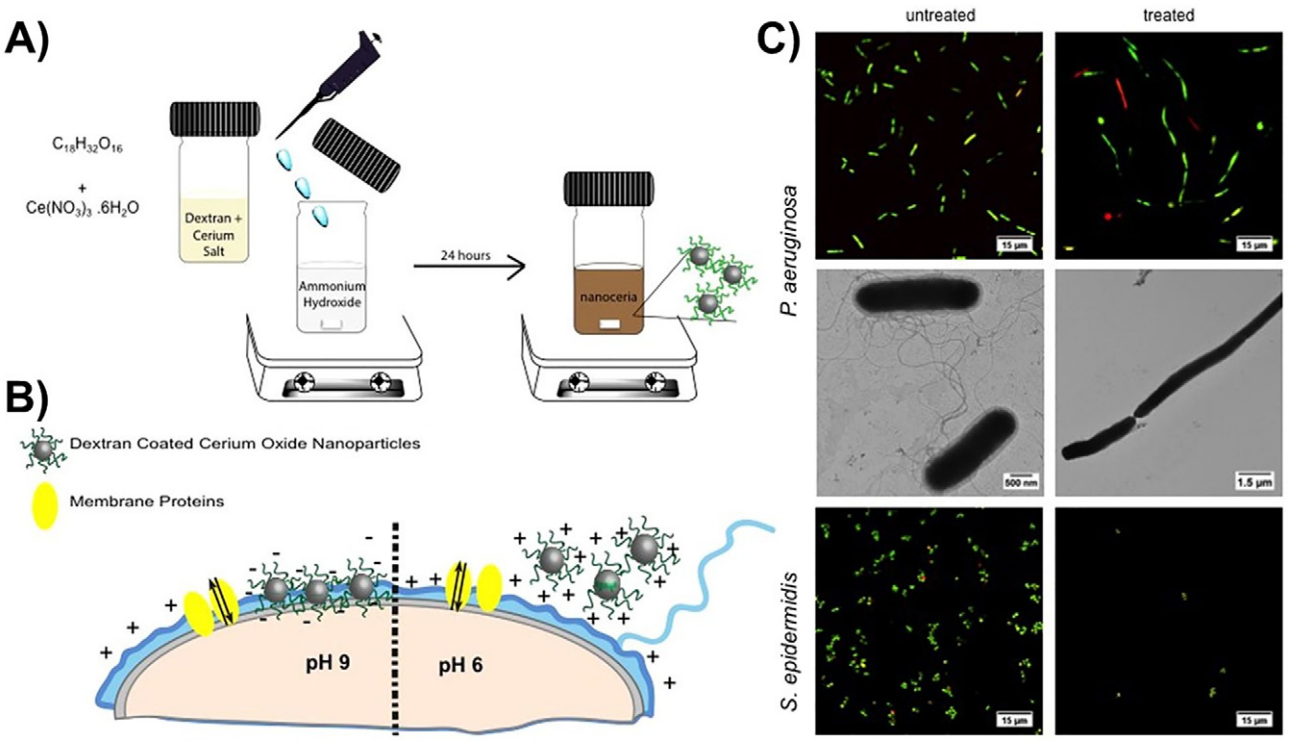

Figure 7. A) The synthetic route for the preparation of the nanoceria. B) The interaction between the positively and negatively charged nanocarrier particle and the positively charged cell membrane. C) LIVE/DEAD staining and TEM imaging of a 10^6 CFU/mL culture of Gram-negative bacteria *P. aeruginosa* and Gram-positive bacteria *S. epidermidis* after 6 h at pH 9, treated and untreated with nanoceria. Reproduced with permission.[76] Copyright 2019, Springer Nature Publishing AG.

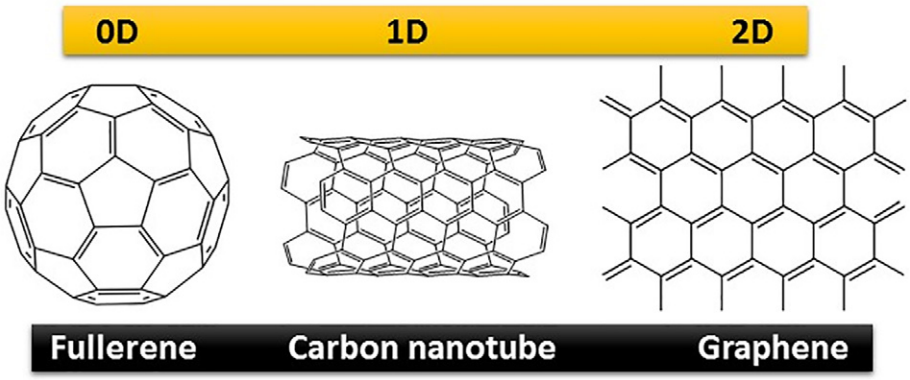

Figure 8. Examples of different carbon nanostructures, fullerene, carbon nanotube (CNT) and graphene.

decolorization step to remove leftover pigments, providing a colorless product (Figure 10, *A*).[115] Chitosan, the most important chitin derivative in terms of medical applications, can be obtained by partial deacetylation of chitin under alkaline conditions.[115] Currently, work has been performed to enhance the antibacterial activity of chitosan by introducing alkyl groups to the amine groups affording a quaternized *N*-alkyl chitosan derivative (*N*,*N*-dimethyl chitosan (DMC), *N*,*N*,*N*-Trimethyl chitosan (TMC)), or modifying with phenolic hydroxyl moieties (Figure 10, *B*). In this context, with the alkyl moiety attached, generating the quaternized *N*-alkyl chitosan also influence the activity. In general, increasing the alkyl chain display higher activity. Additionally, the molecular weight and degree of acetylation are other important parameters impacting antimicrobial effectiveness, where in general a lower molecular weight and degree of acetylation promote better efficiency.[116] A very promising water-soluble quaternized chitosan derivative, hydroxypropyl trimethyl ammonium chloride chitosan (HACC), obtained from the reaction of chitosan with glycidyl trimethylammonium chloride, has attracted much attention due to its strong antibacterial and broad spectrum activity.[117,118] Wang and co-authors have incorporated HACC into biomimetic calcium phosphate (BioCaP) granules containing BMP-2 to act as a bone-

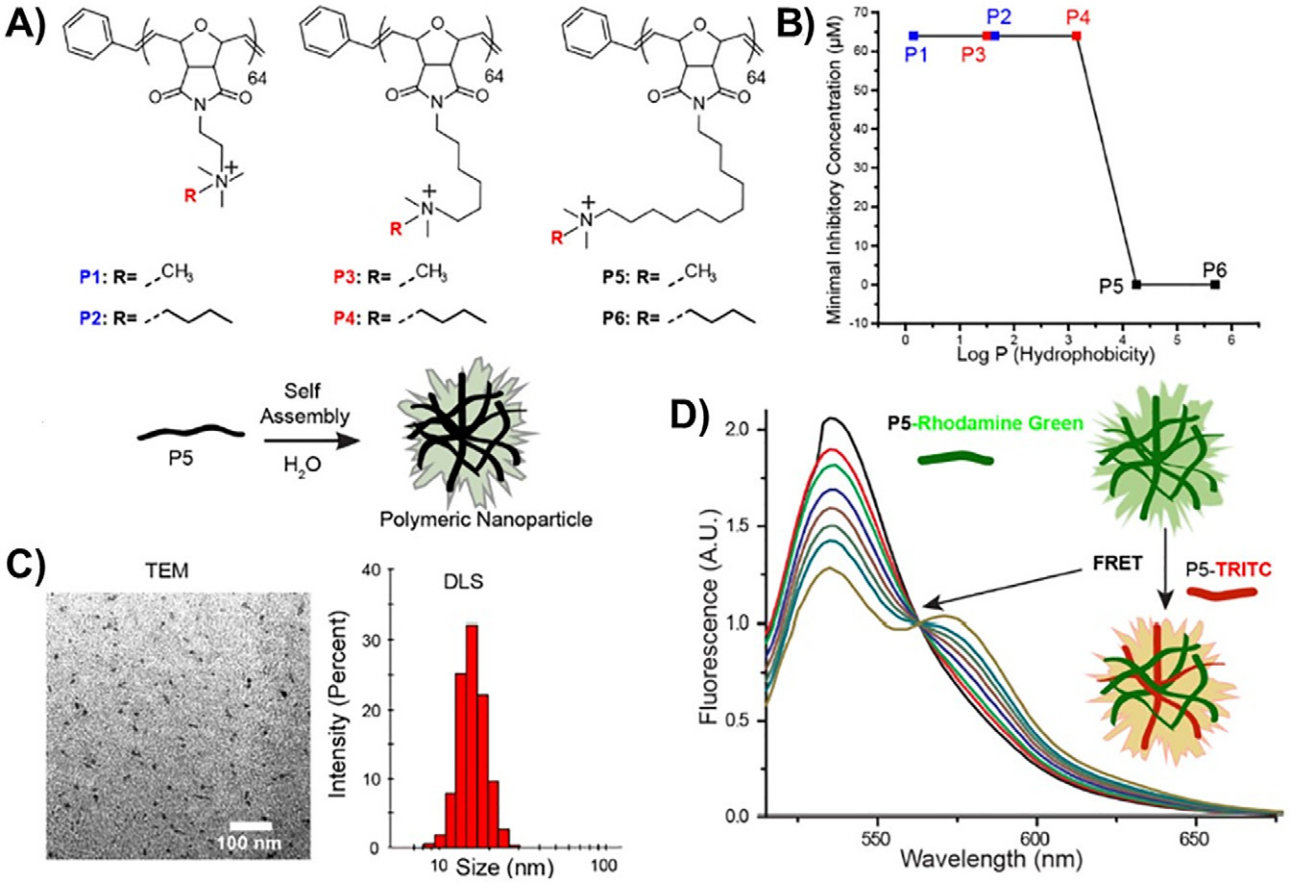

Figure 9. A) Molecular structures of oxanorbornene polymer derivatives. B) Minimal inhibitory concentration as a function of calculated hydrophobic values of polymer derivatives. C) Scheme, transmission electron microscopy (TEM) image and dynamic light scattering (DLS) plot of P5-homopolymers self-assembly structure. D) Förster resonance energy transfer (FRET) results between P5-Rhodamine Green and P5-TRITC indicating the formation of polymeric nanoparticles. Reproduced with permission.[95] Copyright 2018, American Chemical Society.

defect-filling material.[119] The BMP-2-BioCaP/HACC complex was able to rapidly kill site-infection associated bacteria and thereafter induce new bone formation.[119] The antibacterial mechanism of action of chitosan proceeds through several pathways.[116] The positively cationic chitosan interacts with the negatively charged bacterial cell surface through ionic interaction leading to cell wall leakage by changing the membrane permeability and provoking internal osmotic imbalance.[120] This mechanism can also lead to the leakage of intracellular electrolytes by hydrolysis of the peptidoglycans in the bacterial wall.[121] A second mechanism of action could be made by the penetration of chitosan into the nuclei and thereby inhibiting mRNA and protein synthesis. The third suggested pathway is through the formation of an external barrier and further chelating metals and thereby hampering the elimination of essential nutrients vital for bacterial growth.

Antimicrobial peptides (AMPs) comprise a class of peptides with a small size (<10 kDa), cationic and amphipathic properties, and with various length, sequence and structure, that are part of the innate immune system of most living organisms against invading pathogens.[12] Several AMPs have been isolated through the years from a wide variety of animals, plants, bacteria and fungi, and they have emerged as an alternative to conventional antibiotic therapy. They have demonstrated high therapeutic indices (TI, selectivity toward bacterial cells calculated as HC50 (hemolytic activity)/MIC) of ~900 and ~330013 against planktonic bacteria and a broad spectrum of activity against microorganisms including Gram-positive bacteria, Gram-negative bacteria, antibiotic-resistant bacteria, protozoa, yeast, fungi and viruses.[12,95] The AMPs are classified based on their 3D structure into five groups: α-helical AMPs, cysteine rich AMPs, β-sheet AMPs, AMPs rich in regular amino acids and AMPs with rare modified amino acids.[12] The antimicrobial action of most of these peptides are based on the amphipathic structure of the AMPs that is able to bind to the negatively charged outer surfaces of microorganisms and to disrupt and permeate their cell membranes, leading to the lysis of the cell.[12] Eckhard et al. have tested four ultra-short lipopeptides (C16-KGGK, C16-KLLK, C16-KAAK and C16-KKK) and an amphipathic α-helical antimicrobial peptide (Amp-1D) against *E. faecalis*, a multidrug resistant nosocomial pathogen and a persistent pathogen in root canal infections.[122] Among the five tested AMPs, C16-KGGK was the most effective, and consequently it was used to produce biohybrids (polymer-antimicrobial peptide) with two types of polymers: poly(lactic acid-*co*-castor oil) (DLLA) and ricinoleic acid-based poly(ester-anhydride) P(SA-RA).[122] The antibacterial activity of the biohybrids were tested and the results showed that

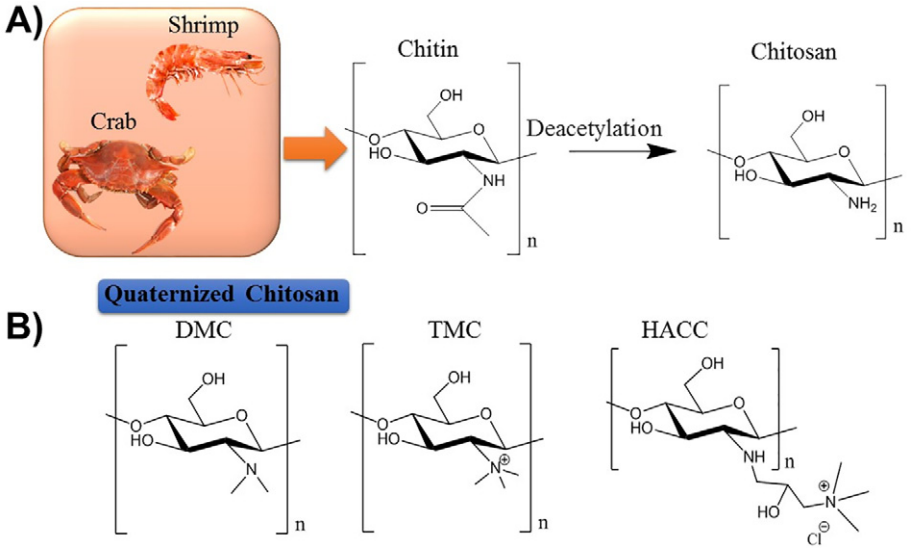

Figure 10. A) The source of chitin and chitosan and their chemical structures. B) Quaternized chitosan derivatives: *N*,*N*-dimethyl chitosan (DMC), *N*,*N*,*N*-Trimethyl chitosan (TMC) and hydroxypropyl trimethyl ammonium chloride chitosan (HACC).

they exhibited strong and improved activity against *E. faecalis* and therefore these materials can be advantageous in root canal treatment for the prevention of endodontic failure.[122] Moreover, natural polyphenols are ubiquitous products widely found in nature, e.g. in fruits, nuts, seeds, stems and flowers, and have been shown to display antibacterial properties.[13,123] In this context, polyphenols[124] such as lignin[125–127] and tannin[128,129] comprising of gallol and catechol functionalities are interesting types of molecules with activity against a wide spectrum of microbials.[130] These components could serve as an important approach for inventing novel type of antimicrobial agent against clinically multidrug-resistant microorganisms (Figure 11).[131]

Osteoinductive biomaterials

Effective materials for the replacement of bone tissue have mimicked natural bone in terms of composition and/or surface structure.[19] Bones are formed by nano, micro and macro porous structures composed essentially of an extracellular matrix (ECM), mainly collagen disposed in nanofibers, and crystals of HA: $Ca_{10}(PO_4)_6(OH)_2$ oriented in the same direction as the collagen fibres.[19,132] The composite structure of mineralized collagen nanofibers (fibrils) aligned and organized to form higher order structures eventually lead to the formation of complete bone.[132] Here, CaPs, such as HA (is relatively insoluble, resorbs slowly and undergoes lower conversion to a bone-like material after implantation), β-TCP: $Ca_3(PO_4)_2$ (more soluble, general lower mechanical strength than HA) and BCP (BCP: a combination of HA and β-TCP), present good biocompatibility, osteoconductivity and osteoinductivity due to their similarity to the inorganic composition of bone.[19,133,134] In general, CaPs display high compressive strength and low ductility, providing high resistance towards deformation but, at the same time, with brittleness.[135] An alternative approach that has been explored is the incorporation of these CaPs as a bioactive load into biodegradable polymeric matrices, such as chitosan,[136] collagen,[137,138] poly(-caprolactone) (PCL),[135] PLA and PGA,[139] and other polymers that have been used in medicine for drug delivery or therapeutic systems.[140,141] Caballero and co-workers have developed a chitosan hydrogel with intertwined apatite particles to meet the bio-physical and mechanical properties required for a potential bone substitute.[136] The biomaterial is employed as ink, composed of suspensions of CaPs particles in an acidic aqueous solution containing chitosan, and further used for producing scaffolds using 3D printing by a robocasting approach. The results indicated that the rheological properties of the ink can be modulated through controlling the formulation of the suspensions, such as chitosan concentration and inorganic to organic ratio.[136] Likewise, BGs, another class of inorganic materials, have been extensively studied for bone tissue engineering applications.[142] The mechanism of the BG bioactivity is based on its ability to form a carbonate-substituted HA like (HCA) layer on the glass surface when in contact with body fluids, and due to the similarity to the HCA layer of the mineral constituent of bone, it binds firmly with living bone and tissues.[134] There is a growing interest in the application of BG scaffolds for bone tissue engineering, due to their bone bonding ability, osteoconductivity and osteoinductivity.[140] Plenty of reviews reporting the various applications of BG for bone tissue engineering can be found in the literature.[134,143,144] Chen and co-workers obtained for the first time, a 3D highly porous, mechanically strong, bioactive and biodegradable scaffold by the replication technique using 45S5 BG powder.[145] After immersion in simulated body fluid (SBF) for 28 days, its crystalline phase ($Na_2Ca_2Si_3O_9$) transformed into an amorphous bioactive and biodegradable CaP phase.[145] Apart from 45S5 Bioglass®, several other silicate glasses have been developed and commercialized.[146,147] Recently, the application of bioactive silicate nanoplatelets, such as Laponite® ($Na^+_{0.7}[(Mg_{5.5}Li_{0.3})Si_{0.8}O_{20}(OH)_4]^-_{0.7}$),[148,149] have shown great interest due to their wide range of properties such as osteoinduction property.[150] They have been shown to successfully trigger the osteogenic

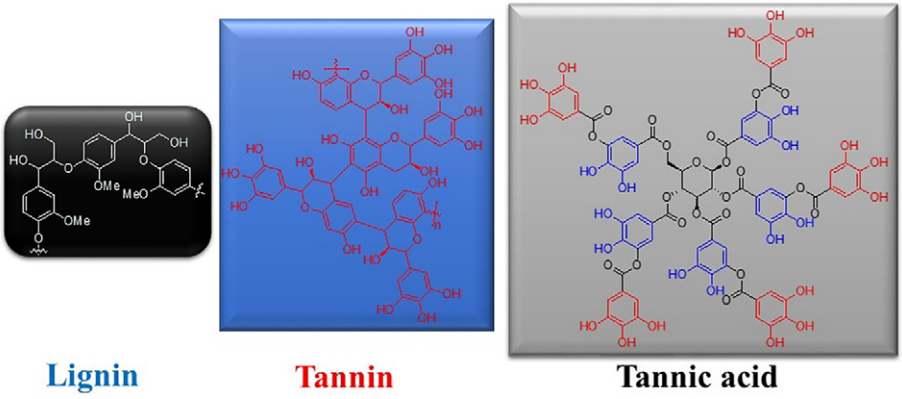

Figure 11. The chemical structure of the polyphenols lignin, tannin and tannic acid.

differentiation of human mesenchymal stem cells (hMSCs),[151] and therefore demonstrated as a promising candidate for various orthopaedic applications.[152–154] Furthermore, several elements such as Mg, Zinc (Zn), Sr, silicon (Si), selenium (Se) and Cu, have shown influences on the proliferation and differentiation of osteoblasts. Here, Mg is a biocompatible, biodegradable and naturally occurring element in the human body (0.4 g kg^{-1}), mostly in bones where Mg cations (Mg^{2+}) are located at the edges of apatite minerals. It play an important role in the structure and density of bone apatite and consequently in establishing bone mechanical properties.[155] Additionally, the Zn plays an important role in various physiological processes, such as cytoskeleton maintenance, immune function, cellular signalling, and synthesis of about 1400 zinc-finger proteins.[133] Here, Webster and co-authors reported that doping of HA with zinc ions (Zn^{2+}) significantly increased osteoblast adhesion compared to the non-doped material,[156] as well as increased osteoblast alkaline phosphatase (ALP) activity and Ca-containing mineral deposition.[157] Moreover, Sr influence bone compressive strength,[133] and promotes apoptosis (cell death) of osteoclasts (a cell whose function is the resorption of the bone matrix), favours the osteogenic differentiation of MSCs and accelerates bone formation *in vivo*.[158,159] Due to its osteoinductive properties, Sr has been incorporated into BGs,[160] CaPs,[161] and directly in nanocomposites.[162] On the other hand, Si is essential for metabolic processes, formation and calcification of bone tissue. In addition to increasing bone mineral density and stimulating the formation of collagen and osteoblastic differentiation.[163–167] Moreover, Se has an essential function in antioxidant defence mechanisms supporting immune surveillance and cell proliferation and differentiation.[168] Several Se-proteins are known to be expressed in human fetal osteoblasts, and these proteins contribute to the protection against ROS, reactive nitrogen species (RNS) and oxidative damage.[168–170]

Cu is a bioactive element reported to have hypoxia-mimicking capacities, leading to upregulation of genes and thereby enhancing angiogenesis.[143,171] Shi and co-workers have loaded mesoporous silica nanospheres (MSNs) with Cu to induce osteogenic differentiation from bone MSCs (BMSCs) (Figure 12).[171] Cu-MSNs were produced by one-pot synthesis which involved mixing CTAB, ammonium fluoride (NH_4F), tetraethoxysilane (TEOS) and copper nitrate trihydrate ($Cu(NO_3)_2.3H_2O$), in an ethanol solution. Cu-MSNs were phagocytized by immune cells and led to osteogenic differentiation of BMSCs via the activation of the Oncostation M (OSM) pathway.[171] All five genes (ALP, osteopontin (OPN), osteocalcin (OCN), COL1, and integrin-binding sialoprotein (IBSP)) tested were upregulated by the presence of Cu-MSNs compared to MSNs controls, and in addition, MSNs containing Cu presented a higher level of mineralization.

Also, osteogenic growth factors (such as fibroblast growth factor (FGF), TGF, vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF)) have been used to improve osteoinductivity properties of materials used to replace bone.[21] During osteogenesis (that is, the bone formation process), several growth factors are secreted by osteoprogenitor cells and osteoblasts to recruit MSCs and induce osteoblastic differentiation. Therefore, the addition of these growth factors, aim to accelerate the osseointegration process.[21] Among these growth factors, BMPs, which belongs to the family of TGF proteins, is the type of growth factors most commonly used to improve osseointegration, and the FDA has granted approval for clinical applications of these components.[20] In addition to growth factors, some peptides have been used to accelerate the osseointegration process, such as the arginine-glycine-aspartic acid (RGD) containing peptides, parathyroid hormone (PTH) ($PTH_{1–34}$ and PTHrPs), thrombin peptide 508 (TP508), osteogenic growth peptide (OGP), PepGen (P-15), calcitonin gene-related peptides (CGRP) and ECM-derived peptides. These promote the adhesion and differentiation of osteoblasts aside from having other potential effects on bone regeneration.[20,21,172]

Dual functional biomaterials

The design and development of innovative dual functional biomaterials, having the ability to both promote osteoinduction and at the same time possess antimicrobial activity, thus preventing any infection, is a challenging task, nevertheless, it is vital and beneficial for various orthopaedic applications.[1] However, one has to bear in mind that such biomaterials should also display additional properties such as good biocompatibility, sufficient mechanical strength and proper degradability in order to be

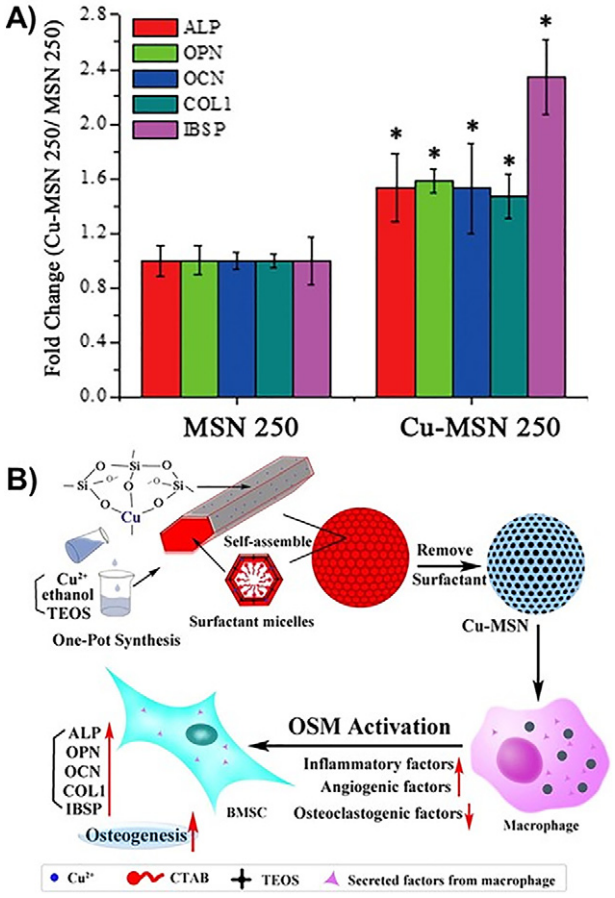

Figure 12. A) Upregulation of osteogenic genes (ALP, OPN, OCN, COL1, IBSP). B) Scheme representing (i) the synthesis of Cu-MSNs by a simple one-pot approach in which the Cu-complexes self-assembled with the structure-directing agent (CTAB), and (ii) the action of Cu-MSNs that after being phagocytized by macrophages, induce a beneficial immune response and stimulate osteogenic differentiation from BMSCs. Reproduced with permission.[171] Copyright 2015, Acta Materialia Inc. Published by Elsevier Ltd.

clinically relevant. Moreover, in the case of the co-delivery system of the antimicrobial and osteoinductive components, they should be delivered locally and in sustained and prolonged release either simultaneously or in a sequential manner.[173] There are several ways of combining the two components with the biomaterials depending on the desired application, for instance through superficial adsorption, chemically bonded, internal encapsulation/physically entrapment or through coating a layer.[1] In this context, Lobo et al. recently demonstrated the design and fabrication of an electrospun nanofibers blend based on the merging of three biomaterials, PCL, PEG and gelatine methacryloyl (GelMA), electrospinning and then further double crosslinking (glutaraldehyde- and UV-crosslinking) providing a material with improved mechanical-, wettability and biological performance compared to solely PCL nanofibers (Figure 13, *A*).[174] The group suggested their fabricated nanofiber as a potential material for bone tissue regeneration based on several findings, the upregulation of human osteoblasts (hFOB), the enhanced osteoblast ALP activity (by 10-fold at day 14 compared to pure PCL fibres) and Ca deposition (by 1.3-fold at day 21 compared to pure PCL fibres). Additionally, the same group further investigated the bactericidal activity of their devised electrospun nanofibers against some of the three most common bacteria, *S. aureus*, *P. aeruginosa* and MRSA.[175] After crosslinking the nanofiber blends, it displayed good antibacterial activity against all three of the bacteria tested, besides being compared solely to PCL fibres, the blend showed a 10-fold reduction of *S. aureus* and *P. aeruginosa*, which is more than a 90% bacteria reduction (Figure 13, *B*).

Kumar et al. employed a different strategy for improving the mechanical properties of the PCL composites and further promoted osteogenesis and bactericidal properties of their devised material.[176] The authors designed carboxyl- and amine-functionalized multi-walled CNTs (MWCNTs) for better integration with the PCL and fabricated the composites through a melting and mixing approach. The composite functionalized with amine groups showed the best results with significant improvements over pure PCL regarding mechanical properties, osteoblast proliferation, mineralization and bacterial resistance. Moreover, Li et al. devised a synthetic bone graft made from poly(2-hydroxyethyl methacrylate)-nanocrystalline HA (pHEMA-nHA).[177] The authors successfully encapsulated the osteoinductive growth factor recombinant human BMP-2 (rhBMP-2) and the antibiotic vancomycin without impeding the mechanical properties of the material. The study confirmed the ability of the bone graft to release both components in a sustained and localized manner. Vancomycin showed a release of 35-50% in the first 2 days and then a further sustained release over 2 weeks. The antibacterial activity of the antibiotic released was demonstrated by the inhibition of *E. coli*. Moreover, rhBMP-2 displayed a sustained release for over 1 week with good osteogenic trans differentiation ability in cell culture. BMP-2 has also been combined with AgNPs in a PLGA composite graft to induce bone repair in rat femoral infected segmental defects.[178] The graft loaded with AgNPs at 2.0 % showed complete inhibition of *S. aureus* (at an inoculum of 10^7 colony forming units (CFU)) *in vitro*. Also, the *in vivo* experiments showed good performance where only limited bacterial colonies were detected, and instead, more red blood cells were observed after 2 weeks of implantation. PCL electrospun incorporated with ZnO-NPs has been disclosed as an osteoinductive and antibacterial composite, and also as a potential candidate for periodontal (tooth-supporting tissue) tissue engineering.[179] The ability of the material to promote bone growth was investigated in a rat periodontal defect model (Figure 14, *A*). The *in vitro* biological performance of the membrane was investigated on human periodontal ligament stem cells (PDLSCs). Cell proliferation assays confirmed that having 0.5% (w/v) of ZnO provided good cell proliferation, whereas 1% significantly decreased cell viability. This was explained due to the high amount of ZnO promoting the production of hydrogen peroxide (H_2O_2) resulting in higher levels of ROS. Moreover, the material also displayed high ALP activity and significant bacteria reduction of *Porphyromonas gingivalis* (*P. gingivalis*). Additionally, the *in vivo* results after 6 weeks displayed promising results based on the decreased distance between the cementoenamel junction (CEJ) and the bone crest (Figure 14, *B*). Nevertheless, additionally, *in vivo* characterization is needed to fully understand the *in vivo* performance of the membrane.

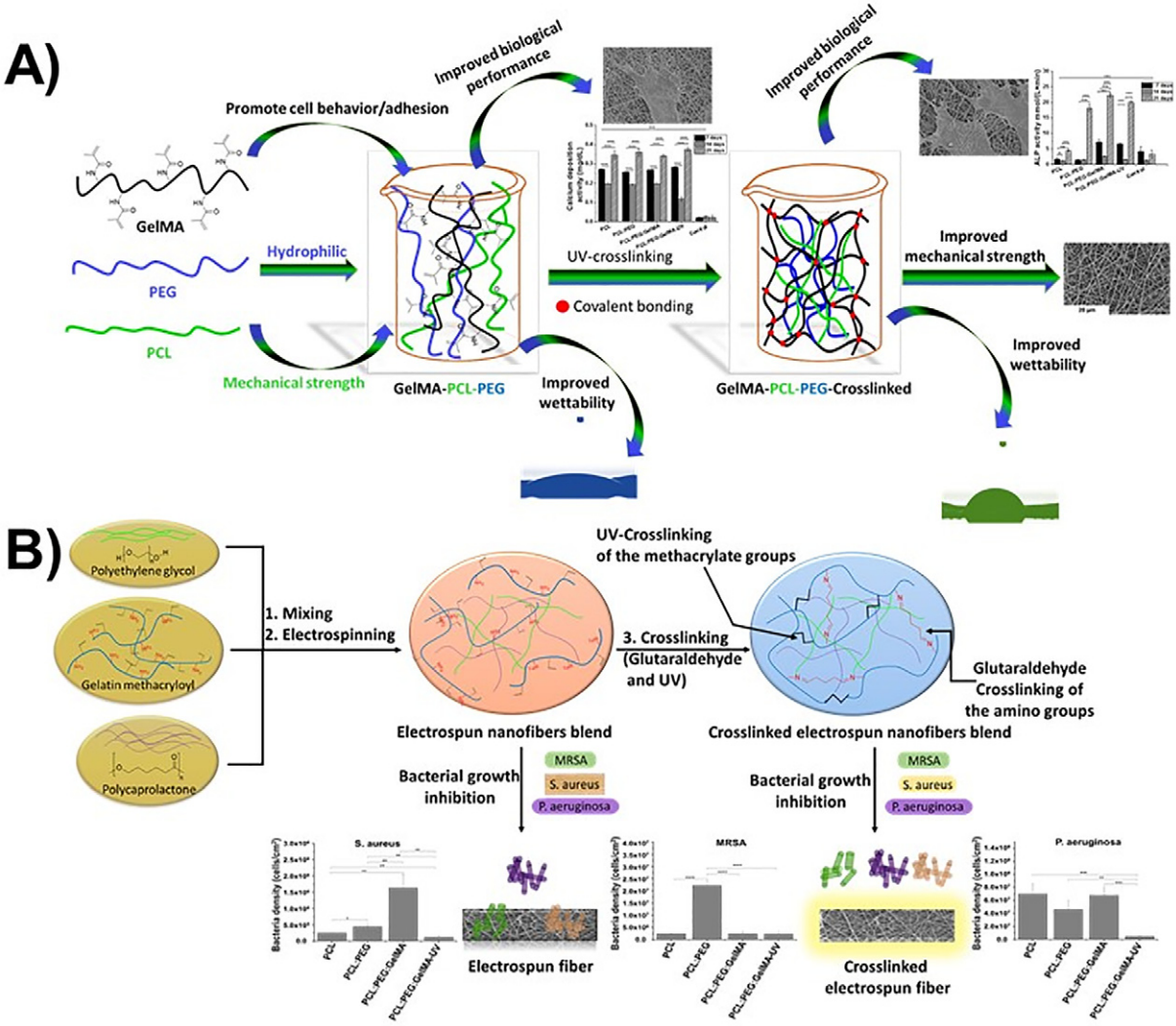

Figure 13. A) The design and fabrication of an electrospun nanofiber blend through the combination of the biomaterials polycaprolactone (PCL), polyethylene glycol (PEG) and gelatin methacryloyl (GelMA), employing electrospun technology and subsequently double crosslinking (first glutaraldehyde- and then UV-crosslinking). The electrospun nanofiber blend displayed improved wettability, mechanical strength and biological (osteoinduction) performance. Reproduced with permission.[174] Copyright 2018, Lobo et al. Dove Medical Press Limited. B) The bactericidal activity of the electrospun nanofibers against *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Methicillin resistant Staphylococcus aureus* (MRSA). Reproduced with permission.[175] Copyright 2018, De Pula et al.

Chitosan has been shown to be a promising biomaterial for bone tissue engineering applications, in fact, in some studies, it has been shown to have an osteoinducing effect.[180] A multifunctional chitosan-45S5 BG-poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) microsphere (CS-BG-MS) composite membrane was developed for bone regeneration.[181] The incorporation of the various components into the system provided favourable properties, such as increased surface roughness (0.20 ± 0.02 of only CS membrane and 0.7 ± 0.2 of CS-BG-MS), improved hydrophilicity (from contact angle (CA) 103^{o} ± 5 to 63^{o} ± 4), decreased swelling ratio (from ~150% to ~75% after 14 days) and decreased degradation. Moreover, the incorporation of the BG promoted osteoinduction properties of the devised membrane. This was confirmed through the evaluation of osteoblast-like MG-63 human osteosarcoma cells showing a significant increase in ALP activity after 14 days. Importantly, the antibacterial drug tetracycline hydrochloride (TCH) was successfully loaded into the system and was delivered in a sustained and prolonged release compared to the control (CS membrane). The control showed a complete release after 24, whilst the system displayed a prolonged release for a period of 7 ~days, with an initial release of 60% at day 1. This property provides a system with a dual effect. Moreover, calcium sulphate ($CaSO_4$) cement composites comprised of the antibiotic drug vancomycin and rhBMP-2 have also been demonstrated for the treatment of rabbit tibial defects.[182] The *in vivo* results showed an initial burst release at day 1 of both the drugs and then a further sustained release for more than 14 and 28 days, respectively. Interestingly, the control showed more bone formation at day 14 than the implant containing both the antibiotic drug and the growth factor. However, significant new bone formation 14 days after implantation was observed, whilst

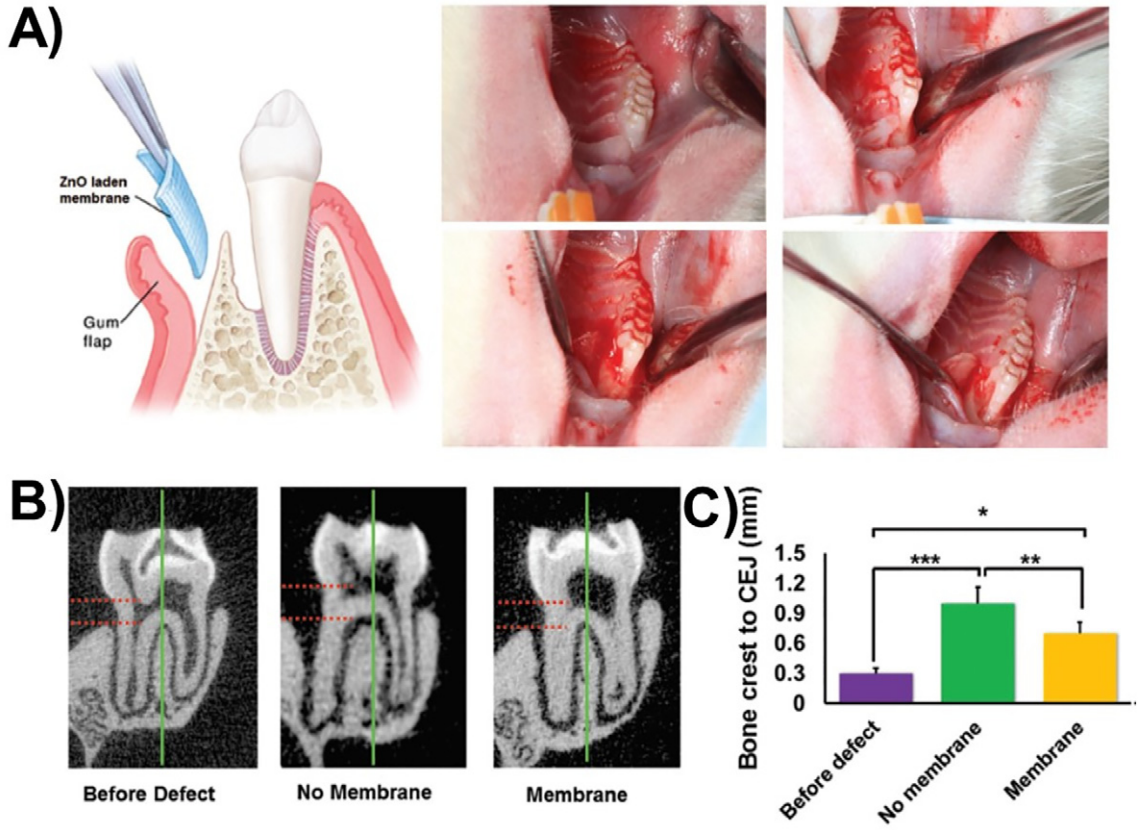

Figure 14. A) Schematic of the rat periodontal defect model and treatment employing electrospun polycaprolactone (PCL) and loaded with zinc oxide nanoparticles (ZnO-NPs) and images from the *in vivo* experiments. B) The micro-CT analysis of the rat maxilla displaying the control, defected area and the treated defect with the electrospun PCL. C) Semi-quantitative analysis of the *in vivo* study. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Reproduced with permission.[179] Copyright 1999–2019, John Wiley & Sons, Inc.

the control did not show any more bone formation. Moreover, several other biomaterials have been disclosed as a co-delivery system for the bone defect and infection treatment, such as a collagen scaffold[183] for the co-delivery of AgNPs and BMP-2, and silica-CaP-nanocomposite for the delivery of vancomycin and rhBMP-2.[184] Additionally, stainless steel was coated with AgNPs/PLGA for inducing antimicrobial and osteoinductive properties.[185] Similarly, the nanosphere composite was designed based on PLGA/AgNPs-PGA and ascorbic acid (AscH), whereas the AscH further added antioxidant properties into the system.[186] As *vide supra* described, Sr has the ability to promote bone regeneration, therefore, the co-loading of Sr and Ag into NTs provides both osteoinductive and antibacterial properties. This strategy was applied for the treatment of Ti implants[187] and was shown to repair damaged cortical bone and promote the formation of new bone in both osteoporosis and in rat femoral defect models.[188] Moreover, the *in vitro* antibacterial study showed excellent antibacterial activity against MRSA, *E. coli* and *methicillin-sensitive S. aureus* (MSSA) with no cytotoxicity.

Applications of antimicrobial and osteoinductive biomaterials

As elucidated in this chapter, biomaterials scientists aim to establish coherent associations relating *in vivo* physiological occurrences to implant properties. Furthermore, the potential role for BG, ceramics, polymers, metals, and composites as principal biomaterial constituents has been examined. The principles approbated from decades of research have been adopted and adapted by various groups to design orthopaedic implants that are antimicrobial and osteoinductive. Numerous implant configurations have been devised, some of which have been evaluated and approved for clinical applications by government agencies across the globe.[59] Significant studies in the design of dual functional biomaterials, their fabrication technologies, components and applications discussed in this section are summarized in Table 3. Various strategies for the delivery of the active agents have been utilised such as dual-functionalized coating,[189,190] co-delivery vehicles and sequential release substrate loaded with bioactive and antibacterial materials such as BMP-2, Vancomycin and AgNPs, where the base material have been fabricated from material such as polyurethane,[191] CaP[182] or collagen.[183] They all demonstrated their strengths and limitations for instance, despite the collagen based biomaterials showed to promote BMSc differentiation to osteoblasts, it lacked adequate mechanical strength for bone application.[183] On the other hand, the CaP based scaffold demonstrated prolonged release of the agents for more than 21 days, however, initially a burst release was observed. These demonstrated that the technologies need further fine-tuning in order to be smoothly translated into clinical

Table 3
Antibacterial and osteogenic scaffold, their fabrication approaches, constituents and applications.

Mode of action	Scaffold material	Encapsulation agents	Incorporation method	Results	References
Co-delivery substrate	Biodegradable polyurethane	1. BMP-2 2. Vancomycin	Direct addition of reactive mixture	Promote new bone formation and reduce infection. Controlled and sustained release of BMP-2 and release of vancomycin for more than 8 weeks	[191]
	Calcium sulphate	1. BMP-2 2. Vancomycin	Direct addition of reactive mixture	Tested in a rabbit tibial defect model. Demonstrated concurrently delivery. However initial burst release was observed, followed by gradual release for more than 14 and 21 days.	[182]
	Collagen	1. BMP-2 2. AgNPs	Reduction of silver nitrate in collagen to form AgNPs, followed by direct addition of BMP-2 to reactive mixture	Enhanced BMSCs differentiation to osteoblasts. However, lack sufficient mechanical strength.	[183]
Sequential release substrate	Poly(2-hydroxyethyl methacrylate)-Nanocrystalline hydroxyapatite	1. BMP-2 2. Vancomycin	Vancomycin encapsulation and physical adsorption of BMP-2	Sustained and localized delivery for over 2 weeks of vancomycin and over 8 days of BMP-2. The material induced osteogenic transdifferentiation of C2C12 and the antibiotic inhibited *E. Coli*.	[177]
	Poly(*D*,*L*-Lactic-*co*-glycolic acid) (85:15)	1. BMP-2 2. AgNPs	AgNPs encapsulation and physical adsorption of BMP-2	The bone graft demonstrated healing of infected femoral defects in 12 weeks without any traces of bacteria.	[178]
	Zein	1. BMP-2 2. HACC 3. SBA-15	Direct addition of HACC and SBA-15, followed by physical adsorption of BMP-2	Antibacterial activity against S. aureus and E. Coli up to 5 days. BMP-2 release for more than 27 days. Almost fully recovered critical sized radius bone defect after 12 weeks.	[192]
Dual-functionalized substrate coating	Nanopatterned Titanium	1. Polydopamine 2. AgNPs	Polydopamine film deposition on substrate by treatment in dopamine hydrochloride, followed by immersion in silver nitrate and AgNP physical adsorption	Nano-patterned topography for improved bacteria inhibition and enhanced osteoblast ALP activity	[189]
	Titanium	1. Heparin 2. Chitosan 3. HU-308	Layer-by-layer deposition of heparin and chitosan, followed by physical adsorption of HU-308	A sustained release of the HU-308 for over 30 days	[190]

applications. Alternative to co-delivery for certain biomedical challenges could be the application of sequential release where one of the agents is released initially followed by the second one.[177,178,192] Furthermore, insulin-like growth factors (IGFs), quaternized chitosan, BMPs, and AgNPs, among other antibacterial or osteogenic agents, have been extensively explored as biomaterial constituents.[1] Biomaterials for restoring infected bone defects, or for filling defect sites that bear the potential for future contamination or biofilm formation, are marketable as co-delivery systems. In line with these principals, various scaffold configurations including bioactive add-ins have been assayed. For instance, in isolated studies conducted by Guelcher et al. and Wang et al., bone growth scaffolds made from biodegradable polyurethane and $CaSO_4$, respectively, were supplemented jointly with BMP-2 and the potent antibiotic vancomycin.[182,191] *In vivo* results were presented, which corroborated substantial improvements in bone formation due to the co-delivery scaffolds. Guelcher et al. further documented the infection-reduction observed in rat models that received polyurethane treatments supplemented with vancomycin and varying concentrations of BMP-2. It was observed that a higher BMP-2 concentration correlated with an increase in the bacteriostatic potential of the scaffolds.[191] Overall, the porous structure of the polyurethane-based device is preferable to the solid and quick-setting $CaSO_4$. Although inexpensive and osteoconductive, $CaSO_4$ does self-set very rapidly, and this tendency can hinder bone ingrowth and compromise BMP-2 bioactivity.[1]

With regards to the active scaffold constituents, proteinaceous BMP growth factors are well documented to support osteoinductive events, and their orthopaedic medical applications are quintessential.[193] Indeed, demanding medical interventions involving BMP-2 and BMP-7 have been credited with alleviating the complications stemming from spinal defects and non-union fractures.[194] Devices incorporating BMPs have satisfied criteria for clinical applications in the United States including the Infuse™ bone graft collagen sponges manufactured by Medtronic, which are layered with recombinant human BMP-2 by superficial adsorption.[195] Likewise, the Bioventus LLC product, OsteoAMP®, which consists of allogeneic bone with endogenous growth factors including BMP-2 and BMP-7.[196] Vancomycin is principally used to treat intestinal bacterial infections and is often administered as a medication of last resort. With the rise of antibiotic-resistant bacteria, however, agents besides antibiotics are being investigated as bone implant additives. Here, Sun et al. fabricated an AgNP- and BMP-2-encapsulated collagen scaffold intended to support the regeneration of osseous matter in infected wounds.[183] AgNPs have been broadly recognized in medical research for their propensity to aid in wound healing, to ease inflammatory responses, and to restrict the growth of microorganisms.[197] The antibacterial effect as mentioned *vide supra*, is attributed to several key mechanisms that guide wide-spectrum AgNPs toxicity, including the depolarization of bacterial plasma membranes, the induction of intracellular K^+ leakage and ATP depletion, and the inhibition of bacterial cell respiration. Qin and Mahmood et al. further proved independently from their investigations on urine-derived MSCs and murine calvarial pre-osteoblast cells, respectively, that AgNPs administered at carefully regulated concentrations enhanced bone growth and mineralization.[198,199] Their research findings indicated definitively that AgNPs improved actin polymerization and increased the expression of several osteogenic factors.

Revisiting the concept of a dual-functionalized porous collagen devise, as proposed by Sun et al., and considering the foregoing discussion, it is possible to justify the rationale for incorporating AgNPs and BMPs cooperatively into antibacterial bone growth scaffolds. In summary, co-encapsulated collagen matrices were synthesized by the reduction of silver nitrate ($AgNO_3$) in the presence of dissolved collagen templates, followed by direct BMP-2 addition and product lyophilization.[183] A series of *in vitro* assays indicated the efficiency of the BMP-2/AgNP/collagen samples at restricting the growth of vancomycin-resistant *S. aureus*, supporting the proliferation of bone marrow-derived mesenchymal stromal cells, and upregulating the expression of genes (runt-related transcription factor 2/RUNX2, osteopontin, and osteonectin) that are osteogenic modulators.[183]

Nevertheless, the mechanical strength of such a collagen-based device is lacking, and studies must be carried out to improve the basal tolerance of similar frameworks to standard orthopaedic loads. Besides the concerted co-delivery of antibacterial and osteogenic agents, some have loaded therapeutics onto anti-infection bone growth scaffolds using mixed carrying modes. Specifically, the 'mixed carrying modes' concept entails the utilization of dissimilar conjugation strategies, i.e. encapsulation, adsorption, or chemical attachment, for discrete scaffold constituents.[1] The order of the therapeutic unloading or presentation is controllable, and is contingent on the intended principal and secondary scaffold functions. For example, a scaffold with the primary purpose of restoring osseous tissue growth in a defect site may be engineered to release osteoinductive drugs earlier in time, and at a greater rate, than for antibacterial drugs. Re-evaluating the co-delivery configurations described previously, of BMP-2 in combination with vancomycin or AgNPs, alternative systems using mixed carrying modes have been tested and verified for efficacy. In one study, a pHEMA and nano-HA composite were encapsulated with up to a 4.8 wt% of vancomycin and superficially adsorbed by BMP-2.[177] *In vitro* studies performed on mouse myoblast cells (C2C12) had controlled release rates of vancomycin and BMP-2 over 2 weeks and 8 days, respectively, and the data showed exceptional osteogenic differentiation. The same scaffold applied to an *in vivo* rat model yielded full bridging of femoral segmental defects with a bony callus after 8-12 weeks and improved torsional stiffness relative to non-BMP-2 containing controls.[177]

PLGA composites have also been fabricated and incorporated with AgNPs, at a 2.0% effective dose, and physically adsorbed BMP-2.[178] Following their application to *S. aureus*-infected femoral segmental defects in mice, the PLGA-centered scaffolds exhibited excellent osteoinductivity after 12 weeks and stringent bacterial toxicity, without any observed antagonistic effects on pre-osteoblast cells due to the AgNPs.[178] Furthermore, BMP-2 that is superficially adsorbed onto a scaffold surface is often rapidly released, which results in impaired osteoinductivity. One method that has successfully moderated the unloading of adsorbed BMP-2 is the incorporation of mesoporous SBA-15 silica nanoparticles into the scaffold core.[192] As demonstrated in the literature, at increasing concentrations of embedded SBA-15

Table 4
Commonly employed biomaterials and their characteristics, advantages/disadvantages, clinical/preclinical use and FDA approval.

Biomaterial	Osteogenic properties	Bactericidal properties	Characteristics	Advantages	Disadvantages	Comments regarding FDA approval, Clinical and Preclinical uses and commercial products	References
SeNPs functionalized with quercetin and acetylcholine	No	Yes		Synergistically antibacterial performance against MRSA at a low dose	Not supported		89
CeO_2NPs coated with dextran	No	Yes		Antibacterial activity against *P. aeruginosa* and *S. epidermidis*,	Not supported		76
PLGA/SWCNT	No	Yes		Decreased viability and metabolic activity of *E. coli* and *S. epidermidis*	Effect is strongly influenced by SWNT length and concentration; possible toxicity of SWCNT toward cells		103
Quaternary ammonium poly(oxanorborneneimides) polymeric nanoparticles	No	Yes		Eradicate *P. aeruginosa, S. aureus* and *En. cloacae* complex biofilms; low toxicity to mammalian cells; no bacterial resistance toward these nanoparticles after 20 serial passages			95
PHMG/PEG hydrogels	No	Yes		Low toxicity; antibacterial activity against *S. aureus* and *E. coli*; injectable and biodegradable			114
C16-KGGK antimicrobial peptide/poly(lactic acid-*co*-castor oil) and C16-KGGK /ricinoleic acid-based poly(ester-anhydride)	No	Yes	Root canal treatment for the prevention of endodontic failure	Activity against *E. faecalis*			122
Hydroxypropyl trimethyl ammonium chloride chitosan (HACC)/ biomimetic calcium phosphate (BioCaP)/ BMP-2	Yes	Yes	Bone-defect-filling material	Antibacterial activity to MRSA; non-toxic against pre-osteoblasts; BMP2-induced differentiation of pre-osteoblasts; new bone formation was induced in a model of subcutaneous sites in rats			119
Ag/ Hydroxyapatite /Lignin	Yes	Yes	Coating for metallic implants	Antibacterial activity against *S. aureus* TL; non-toxic against healthy immunocompetent peripheral blood mononuclear cells (PBMC); corrosion protection ability			127
Collagen/Hydroxyapatite/β-tricalcium phosphate	Yes	No	Bone filler Synergoss®	The collagen surface layer stimulates bone regeneration; *in vivo* test in a rabbit model shows increased bone volume and mineral apposition		Clinical use	137
Spongious granules from bovine source	Yes	No	Bone filler Bio-Oss®	The granules are purified which makes the material not antigenic and protein free	animal source	Commercial product	137
Xenograft material constituted as a mixture of cancellous and cortical bone (80-20%) from porcine source	Yes	No	Bone filler Gen-Os®	Obtained through a low temperature process which allows to eliminate any pathogenic elements, preserving the structure and the composition of natural collagen and hydroxyapatite	animal source	Commercial product	137
Hydroxyapatite/β-tricalcium phosphate	Yes	No	Bone filler BoneCeramic®	Synthetic source		Commercial product	137
Collagen/hydroxyapatite/α-tricalcium phosphate	Yes	No	Scaffolds for bone regeneration	Presence of macro and micro-pores for fluid exchange and cellular influx; scaffolds implanted into murine femoral defect presented osteoconductive property, with new bone growth incorporating the degrading scaffold materials			138
45S5 Bioglass®	Yes	No	Scaffolds for bone regeneration	3D highly porous, mechanically strong, bioactive and biodegradable scaffold			145
Laponite®	Yes	No		Excellent surface hydrophilicity, serum absorption capacity, cytocompatibility and hemocompatibility; induce osteoblast differentiation of rMSCs and deposition of hydroxyapatite when soaked in SBF; *in vivo* animal implantation demonstrate that Laponite is able to heal bone defect			152
Gelatin/silica	Yes	No		Highly tailorable mechanical property, MSC viability and dissolution characteristic, as function of gelation functionalization with GPTMS			154

(continued on next page)

Table 4 (*continued*)

Biomaterial	Osteogenic properties	Bactericidal properties	Characteristics	Advantages	Disadvantages	Comments regarding FDA approval, Clinical and Preclinical uses and commercial products	References
Silica/poly (γ-glutamic acid)/calcium	Yes	No		Promoted a rapid formation of apatite when immersed in an SBF solution, due to the release of Si and Ca that stimulate bone growth			166
HA doped with Zn^{2+}	Yes	No		Increased osteoblast adhesion, ALP activity and Ca deposition compared to the non-doped material			156, 157
Gelatin/nano-hydroxyapatite/ strontium/ bioglass seeded with BMSCs	Yes	No		High expression level of osteogenic and angiogenic markers; great bone regenerative capacity; the combination of Sr, BG, and nHAp synergistically enhanced the bone regeneration process			162
Copper-doped mesoporous silica nanospheres (MSNs)	Yes	No		Sustained release of both Si and Cu ions; controlled degradability; induced osteogenic/angiogenic factors; suppressed osteoclastogenic factors; osteogenic differentiation of BMSCs			171
PEG/parathyroid hormone peptide/HA/TCP	Yes	No		Stimulated *in situ* bone augmentation in rabbits			172
PCL/PEG/GelMA electrospun nanofibers	Yes	Yes		Improved mechanical property, wettability and biological performance compared to PCL fibers			174, 175
PCL/aMWNTs composites				Improved mechanical property, wettability and biological performance compared to pure PCL			176
BMP-2 and vancomycin encapsulated in pHEMA-nHA synthetic bone +9grafts				Release encapsulated components in a sustained and localized manner	Content of vancomycin impact directly mechanical properties (inversely correlated)		177
BMP-2/NS/PLGA composite grafts				Infected defects healed much faster than infected controls treated without nano silver	Nano silver efficacy is dose dependent		178
PCL/ZnO-NPs electrospun				Tunable mechanical property, degradation rate over to pure PCL	Restricted number of ZnO-NPs		179
Chitosan/Bioglass/PHBV	Yes	Yes	Co-delivery composite membrane used for periodontal regeneration	Improved surface roughness, hydrophilicity, flexibility and degradation rate, capable of releasing drugs of sustained and controlled manner over pure CS membrane	Lower mechanical properties over pure CS	Partly FDA approved, no clinical use, only preclinical, not commercial	181
rhBMP-2 and vancomycin –loaded calcium sulfate com	Yes	Yes	Co-delivery composite cement used for infected bone defects regeneration	Sustained release of antibiotic and growth factors	Initial burst release at day 1 of both the drugs	FDA approved, no clinical use, only preclinical, not commercial	182
BMP-2/AgNPs/Collagen scaffolds	Yes	Yes	Co-delivery composite scaffolds used for infected bone defects regeneration	Improved roughness surface over collagen scaffold	No sufficient mechanical properties for bone repair	FDA approved, no clinical or preclinical use, not commercial	183
rhBMP-2/vancomycin/SCPC75 nanocomposite	Yes	Yes	Co-delivery porous nanocomposite used for infected bone defects regeneration	Sustained release of antibiotic and growth factors, in which is dependent of initial loading composition of SCPC75-Vanc-rhBMP-2	Burst release of vancomycin in the first 8 h	Partly FDA approved, no clinical or preclinical use, not commercial	184
AgNPs/PLGA coated stainless steel	Yes	Yes	Practical, easy to operate and non-toxic silver nanoparticle/PLGA coating method for use in infected bone defects regeneration	Uniform coating formation that prevented bacterial adherence and biofilm formation	Inhibition of bacterial adherence and biofilm formation in a silver proportion dependent manner	FDA approved, no clinical or preclinical use, not commercial	185
Composite PLGA/AgNPPGA/AscH nanospheres	Yes	Yes	The particles were uniform, spherical and have a potential to use in infected bone defects regeneration	capable of delivery ascorbate to the cells having a potential to prevent oxidative stress	The cellular uptake of vitamin C is somewhat limited	FDA approved, no clinical or preclinical use, not commercial	186
Sr and Ag loaded NT structures on titanium surfaces	Yes	Yes	Simple, economical and scalable coating method for titanium dental and orthopedic implants	Sr and Ag release in a controlled and prolonged matter	Burst release of Sr and Ag in first 24 h	Partly FDA approved, no clinical, only preclinical, not commercial	59, 188
Mineralized cancellous bone allograft (MCBA)	Yes	No	Bone filler Puros®	Clinical reports have documented the ability to stabilize implants 5-6 months after grafting.	human source	Commercial product	200

nanoparticles, antimicrobial HACC/zein scaffolds demonstrated a persistent improvement in the sustained release of adsorbed BMP-2.[192] Nevertheless, bulk metal implants have been remodelled in order to support bone growth and to improve anti-infectivity by the introduction of bioactive coatings.

Although the hydrophilicity and roughness of a Ti implant body, for example, may be altered to influence physiologic performance, polymeric and inorganic coatings have been adapted to assist the osteogenic and antimicrobial efficacies of such implants. For example, Zhang et al. demonstrated a novel hierarchical effect in their study of Ti implants that were coated by composites of polydopamine-modified TiO_2 nanotubes and AgNPs.[189] The as-coated Ti implants demonstrated sustained antibacterial activity due to the protracted release of Ag^+, in which the Ag^+ release rate was shortened by the increasing presence of nanotubes. Moreover, the polydopamine and nanotubes layers represented beneficial effects on the *in vitro* growth of mouse osteoblast cells (MC3T3-E1), overall recommending the use of such a coating for antibacterial bone growth scaffold applications.[189] In a similar approach, Qian et al. applied a layer-by-layer deposition routine in order to generate an osteoconductive and antimicrobial Ti implant comprised of a heparin and chitosan coating and adsorbed by the anti-osteoporosis drug HU-308.[190]

Moreover, Table 4 provides a summary of some biomaterials commonly employed in respect to their characteristics, advantages/disadvantages, clinical/preclinical use and FDA approval.

Future perspective and remarks

To date, we have witnessed a plethora of biomaterials with osteoinductive and antibacterial properties. However, very few examples display both properties within the same biomaterial, other than, when employed as a co-delivery system. In particularly, due to the rapid growth of antibiotic resistance microorganisms and its huge impact on global public health, we are urged to solve this enormous challenge. As we have seen, bacterial infection also negatively impacts the healing of bone defects. Nevertheless, the existence of sophisticated chemistries and material designs, combined with inspiration from nature, holds a great future promise for the invention of novel biomaterials overcoming the limitations and challenges we are facing. The ideal biomaterial with dual function should also possess several other properties such as being biocompatible, biodegradable, support tissue attachment, tissue regeneration, cell proliferation, optimal mechanical properties and good integration with the host tissue. Regarding biodegradation, it should perfectly match the healing process of the defect, which could be a huge challenge to predict. Yet, other aspects to consider is also the chemistry employed for the design and fabrication of such a material, which should be in line with a sustainable, facile, scalable and green approach. In this context, through erudite fabrication methodology, there is no need for the employment of an antibacterial agent. Through the fabrication of the biomaterial surface, giving it a specific nanotextured surface, could help prevent bacteria attachment and colony formation. In the case of a co-delivery system, consideration such as local delivery in a sustained and prolonged manner of the antibacterial and osteoinductive agents is vital to overcome challenges associated with cell toxicity. Moreover, avoiding any kind of possible interference between the antibacterial and osteoinductive agent, either during the delivery or during the mechanistic action are eminent. Further future efforts on the fundamental understanding of bacteria and their behaviour, the material of use and the mechanism and interaction between the biomaterial and host tissue are important and will hopefully pave the way developing an arsenal of sophisticated biomaterials with dual function. Here, smart materials may play an important role as an alternative approach to solve some of these challenges. For instance, consider devising a smart material that senses the onset of bacterial infections and thus prevents this from happening immediately, or a smart material knowing exactly when the ultimate task is fulfilled, thus, leaving the host without any trace of being there in the first place. These are some of the future materials we hope will boost this endeavour and advance the current field of dual functioning biomaterials.

Competing interest

The authors do not have any competing interest to disclose.

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